9-15-00

PATENT
Docket No. 2026-4276US1
Express Mail Label No. EL632223629US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UTILITY APPLICATION AND APPLICATION FEE TRANSMITTAL (1.53(b))

COMMISSIONER OF PATENTS
BOX: PATENT APPLICATION
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Named Inventor(s) and Address(es): Masayuki Yanagi, 257 Congressional Lane, #402, Rockville, MD 20852 U.S.A.

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For: CLONED GENOMES OF INFECTIOUS HEPATITIS C VIRUSES AND USES THEREOF

Enclosed are:

[X] 57 page(s) of specification, 1 page(s) of Abstract, 4 page(s) of claims

[X] 49 sheet(s) of drawings [X] formal [ ] informal

[X] 61 sheet(s) of Sequence Listing

[X] 3 page(s) of Declaration and Power of Attorney

[ ] Unsigned
[ ] Newly Executed
[X] Copy from prior application

[X] 1 page copy of Associate Power of Attorney

[X] 1 page copy of Change of Correspondence Address

[ ] Deletion of inventors including Signed Statement under 37 C.F.R. § 1.63(d)(2)

[ ] Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the combined declaration and power of attorney is supplied herein, is considered as being part of the disclosure of the accompanying application and is incorporated herein by reference.

[ ] Microfiche Computer Program (Appendix)

[X] __61__ page(s) of Sequence Listing

[X] computer readable disk containing Sequence Listing

[X] Statement under 37 C.F.R. § 1.821(f) that computer and paper copies of the Sequence Listing are the same.

[ ] Transfer the computer readable disk containing the sequence listing from the parent application to this application.

[ ] Claim for Priority

[ ] Certified copy of Priority Document(s)

[ ] English translation documents

[X] Information Disclosure Statement (3 pages)

[X] Copy of _17_ cited references

[X] PTO-1449 forms (4 pages).

[X] Preliminary Amendment

[X] Return receipt postcard (MPEP 503)

[X] Assignment Papers (assignment cover sheet and assignment documents)

[ ] A check in the amount of $40.00 for recording the Assignment.

[X] Assignment papers filed in parent application Serial No. 09/014,416.

[ ] Certification of chain of title pursuant to 37 C.F.R. § 3.73(b).

[X] This is a [ ] continuation [X] divisional [ ] continuation-in-part (C-I-P) of co-pending application Serial No. 09/014,416.

[X] Cancel in this application original claims 1-41 of the parent application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

[X] A preliminary Amendment is enclosed. (Claims added by this Amendment have been properly numbered consecutively beginning with the number following the highest numbered original claim in the prior application.

[X] The status of the parent application is as follows:

[ ] A Petition For Extension of Time and a Fee therefor has been or is being filed in the parent application to extend the term for action in the parent application until __________________.

[ ] A copy of the Petition for Extension of Time in the co-pending parent application is attached.

[X] No Petition For Extension of Time and Fee therefor are necessary in the co-pending parent application.

[ ] Please abandon the parent application at a time while the parent application is pending or at a time when the petition for extension of time in that application is granted and while this application is pending has been granted a filing date, so as to make this application co-pending.

[ ] Please abandon the parent application at a time while the parent application is pending or at a time when the petition for extension of time in that application is granted and while this application is pending has been granted a filing date, so as to make this application co-pending.

[ ] Transfer the drawing(s) from the patent application to this application.

[X] Amend the specification by inserting before the first line the sentence:
This is a [ ] continuation [X] divisional [ ] continuation-in-part of co-pending application Serial No. 09/014,416.

I. CALCULATION OF APPLICATION FEE (For Other Than A Small Entity)

	Number Filed		Number Extra	Rate	Basic Fee $690.00
Total Claims	26	-20=	6	x$18.00	108.00
Independent Claims	4	- 3=	1	x$78.00	78.00
Multiple Dependent Claims	[X] yes [ ] no		Additional Fee = Add'l Fee =	$260.00 NONE	260.00

Total: $1,136.00

[ ] A statement claiming small entity status is attached or has been filed in the above-identified parent application and its benefit under 37 C.F.R. § 1.28(a) is hereby claimed. Reduced fees under 37 C.F.R. § 1.9(F) (50% of total) paid herewith $ __________.

[X] A check in the amount of $1,136.00 in payment of the application filing fees is attached.

[ ] Charge Fee(s) to Deposit Account No. 13-4500. Order No. ____________. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

[X] The Assistant Commissioner is hereby authorized to charge any additional fees which may be required for filing this application, or credit any overpayment to Deposit Account No. 13-4500, Order No. **2026-4276US1**. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Dated: September 14, 2000

By: Kathryn M Brown
Kathryn M. Brown
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PATENT
Docket No. 2026-4276US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Yanagi et al	Group Art Unit: To be assigned
Serial No.	:	Div. of 09/014,416	Examiner: To be assigned
Filed	:	September 14, 2000	
For	:	CLONED GENOMES OF INFECTIOUS HEPATITIS C VIRUSES AND USES THEREOF	

EXPRESS MAIL CERTIFICATE

Express Mail Label No. EL632223629US

Date of Deposit September 14, 2000

I hereby certify that the following attached paper(s) and/or fee

1. Utility Application and Application Fee Transmittal,
2. Preliminary Amendment,
3. Copy of specification (57 pages), claims (4 pages, 43 claims), abstract (1 page), drawings (49 sheets, formal), Declaration, Associate Power of Attorney, Change of Correspondence Address and Assignment,
4. Substitute Paper Sequence Listing (Exhibit A),
5. Substitute Computer Readable Sequence Listing (Exhibit B),
6. Statement That Content of the Paper and Computer Readable Copies Are The Same (37 CFR §1.821(f) and 1/821(g)) (Exhibit C),
7. Information Disclosure Statement,
8. Form PTO 1449,
9. Copy of 17 references,
10. Check in the amount of $1,136.00, and
11. Return Receipt Postcard

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Commissioner for Patents, Washington, D.C., Box Patent Application, 20231.

Francisco Garcia
(Typed or printed name of person mailing paper(s) and/or fee)

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569802_1

PATENT
Docket No. 2026-4276US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Yanagi et al.	Group Art Unit: To be assigned
Serial No.	:	Divisional of 09/014,416	Examiner: To be assigned
Filed	:	September 14, 2000	
For	:	CLONED GENOMES OF INFECTIOUS HEPATITIS C VIRUSES AND USES THEREOF	

PRELIMINARY AMENDMENT

COMMISSIONER OF PATENTS
Washington, D.C. 20231

Sir:

Prior to examination on the merits, Applicants respectfully request entry of the following Preliminary Amendment.

IN THE SPECIFICATION

On page 1, line 4, after the recitation of “This application”, insert -- is a divisional of U.S. Serial No. 09/014,416 filed January 27, 1998 which --.

On page 9, line 8 after recitation of “sequence” and prior to the recitation of “of a H77C clone” insert -- (SEQ ID NO:2) --.

On page 9, line 9 after recitation of “amino acid sequence” insert -- (SEQ ID NO:1)--.

On page 9, line 29 after recitation of “Figure 7” insert -- A through 7D --.

On page 10, line 20 after recitation of “HVR1” insert -- (SEQ ID NOS:28, 30, 32, 34, 36-38, 41, 43 and 45) --; at line 21 after recitation of “HVR2” insert -- (SEQ ID NOS:29, 31, 33, 35, 39, 40, 42, 44 and 46) --.

On page 10, line 32 after recitation of "1b (pCV-J4L6S)." insert -- 5' UTR for HC-J4 is SEQ ID NO:47, 5' UTR for pCV – J4L6S is SEQ ID NO:48, 5' UTR for pCV-H77C is SEQ ID NO: 49, 3' UTR - 3' variable region for HC-J4 is SEQ ID NO 50 and 53, 3' UTR - 3' variable region for pCV-J4L6S is SEQ ID NO:51 and 54, 3' UTR - 3' variable region for pCV-H77C is SEQ ID NO:52 and 54; 3' UTR - 3' conserved region for H77, pCV-J4L6S and pCV – H77C is SEQ ID NO 55.

On page 12, last line after recitation of Accession No. insert -- 209596 --.

On page 11, line 20 after recitation of "strain HC-J4" insert -- (SEQ ID NO:4) --.

On page 11, line 21 after recitation of "amino acid sequence" insert -- (SEQ ID NO:3) --.

On page 11, line 29 after recitation of "clone pH 77CV-J4" insert -- (SEQ ID NO:6) --.

On page 11, line 31 after recitation of "chimeric 1a/1b clone" insert -- (SEQ ID NO:5) --.

On page 11, line 34 after recitation of "1a infectious clone pCV-H77C" insert -- (pCV-H77C has SEQ ID NOS:56, 57 and 58; pCV-H77C (-98X) has SEQ ID NO:59; pCV-H77C (-42X) has SEQ ID NO:60; pCV-H77C (X-52) has SEQ ID NO:61; pCV-H77C(X) has SEQ ID NO:62; pCV-H77 C(+49X) has SEQ ID NO:63; pCV-H77C (VR-24) has SEQ ID NO:64; and pCV-H77C (-U/UC) has SEQ ID NO:65).

On page 29, Table 1, line 4, after recitation of "H9261F" insert -- SEQ ID NO:7 --; at line 5 after recitation of "H3' x 58R" insert -- SEQ ID NO:8 --; at line 6 after recitation of "H9282F" insert -- SEQ ID NO:9 --; at line 7 after recitation "H3' X 45R" insert -- SEQ ID NO:10 --; at line 8 after recitation of "H9375F" insert -- SEQ ID NO:11 --;

at line 9 after recitation of "H3' X –35R" insert -- SEQ ID NO:12; at line 10 after recitation of "H9386F" insert -- SEQ ID NO:13 --; at line 11 after recitation of "H3' X – 38R" insert -- SEQ ID NO:14 --; at line 12 after recitation of "H1" insert -- SEQ ID NO:15 --; at line 13 after recitation of "H9417R" insert -- SEQ ID NO:17 --.

On page 41, line 1 after recitation of "(5'-GCCTATTGGCCTGGAGTGGTT AGCTC - 3') insert -- SEQ ID NO:18 --; at line 6 after recitation of: AGGATGGCCTTAAGG CCTGGAGTGGTTAGCTCCCCGTTCA - 3')" insert -- SEQ ID NO:19 --.

On page 51, line 1, after recitation of "H2751S (Cla I/Nde I)" insert --SEQ ID NO:20 --; at line 3 after recitation of " H2870R" insert -- SEQ ID NO:21 --; at line 5 after recitation of "H7851S" insert -- SEQ ID NO:22 --; at line 7 after recitation of "H9173 R(P-M)" insert -- SEQ ID NO:23 --; at line 9 after the recitation of "H9140S (P-M)" insert -- SEQ ID NO:24 --; at line 11 after the recitation of "H9417R" insert -- SEQ ID NO:25 --; at line 14 after recitation of "J4-2271S" insert -- SEQ ID NO:26 --; at line 16 after recitation of "J4-2776R (Nde I)" insert -- SEQ ID NO:27 --.

After page 62 of the "Abstract of the Disclosure" insert -- Sequence Listing -- page number 1 through 61.

IN THE CLAIMS

Please cancel claims 1-41 without prejudice.

Please amend the following claims:

42. (Amended) A composition comprising a <u>purified and isolated</u> nucleic acid molecule [of claim 1] suspended in a suitable amount of a pharmaceutically acceptable diluent or excipient<u>, said nucleic acid molecule encodes human hepatitis C</u>

virus, wherein expression of said molecule in transfected cells results in production of virus when transfected into cells.

43. (Amended) A method for treating hepatitis C viral infection comprising the administration to [a] an animal in need thereof of a clinically effective amount of the composition of claim 42.

Please add the following new claims:

-- 44. The composition of claim 42, wherein the molecule encodes the amino acid sequence of SEQ ID NO:3 shown in Figures 14G-14H.

45. The composition of claim 42, wherein the molecule comprises the nucleic acid sequence of SEQ ID NO:4 shown in Figures 14A-14F.

46. The composition of claim 42, wherein the molecule encodes the amino acid sequence of SEQ ID NO:1 shown in Figures 4G-4H.

47. The composition of claim 42, wherein the molecule comprises the nucleic acid sequence of SEQ ID NO:2 shown in Figures 4A-4F.

48. A composition comprising a purified and isolated nucleic acid molecule suspended in a suitable amount of a pharmaceutically acceptable diluent or excipient, said nucleic acid molecule encodes human hepatitis C virus, wherein expression of said molecule in transfected cells results in production of virus, wherein a fragment of said molecule which encodes the structural region of hepatitis C virus has been replaced by the structural region from the genome of another hepatitis C virus strain.

49. The composition according to claim 48, wherein the molecule encodes the amino acid sequence of SEQ ID NO:5 shown in Figures 16G-16H.

50. The composition according to claim 48, wherein the molecule comprises the nucleic acid sequence of SEQ ID NO:6 shown in Figures 16A-16F.

51. A composition comprising a purified and isolated nucleic acid molecule suspended in a suitable amount of a pharmaceutically acceptable diluent or excipient, said nucleic acid molecule encodes human hepatitis C virus, wherein expression of said molecule in transfected cells results in production of virus, wherein a fragment of the nucleic acid molecule which encodes at least one HCV protein has been replaced by a fragment of the genome of another hepatitis C virus strain which encodes the corresponding protein.

52. The composition of claim 51, wherein the protein is selected from the group consisting of NS3 protease, E1 protein, E2 protein and NS4 protein.

53. A composition comprising a purified and isolated nucleic acid molecule suspended in a suitable amount of a pharmaceutically acceptable diluent or excipient, said nucleic acid molecule encodes human hepatitis C virus, wherein expression of said molecule in transfected cells results in production of virus, wherein a fragment of the molecule encoding all or part of an HCV protein has been deleted and, wherein the HCV protein is selected from the group consisting of P7, NS4B and NS5A proteins.

54. The composition according to claims 40 or 48, wherein the nucleic acid molecule encodes an HCV protease selected from the group consisting of NS3 domain protease, NS3-NS4 fusion polypeptide and NS2-NS3 protease.

55. A method of immunizing an animal against hepatitis C virus comprising administration of a composition of claim 42, 48, 51 or 53 in an amount effective to induce immunity against hepatitis C virus.

56. The method according to claim 55, wherein the composition is provided prophylactically.

57. The method according to claim 55, wherein the composition is provided to an animal infected with a hepatitis C virus. --

REMARKS

A restriction requirement was placed on the claims in the parent application Serial No. 09/014,416. Applicants are pursuing herein the claims of Group VII, claims 42 and 43, in the present divisional application.

New claims 44-57 have been added, which find support from the specification and original claims. Claims 44-50 are supported by claims 2-8, respectively. Claims 51-52 are supported by claims 9 and 10. Claims 53-54 are supported by claims 11, 12 and 28. Claims 55-57 are supported by claim 43 and at page 6, lines 16-30 and page 7, lines 4-5.

No new matter has been added by the Preliminary Amendment. Entry thereof is respectfully requested.

Applicants have also filed herein a sequence listing in compliance with the sequence rules under 37 C.F.R. §1.821-§1.825 (Exhibit A), a computer readable sequence listing (Exhibit B) and a statement under 37 C.F.R. §1.821(f) and §1.821(g) which states that the content of the paper sequence and the computer readable sequence listings are identical and that no new matter has been added (Exhibit C).

Entry and favorable action by the Examiner is respectfully requested.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Dated: September 14, 2000

By: Kathryn M Brown
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Title Of Invention

Cloned Genomes Of Infectious
Hepatitis C Viruses And Uses Thereof

This application claims the benefit of U.S. Provisional Application No. 60/053,062 filed July 18, 1997.

Field Of Invention

The present invention relates to molecular approaches to the production of nucleic acid sequences which comprise the genome of infectious hepatitis C viruses. In particular, the invention provides nucleic acid sequences which comprise the genomes of infectious hepatitis C viruses of genotype 1a and 1b strains. The invention therefore relates to the use of these sequences, and polypeptides encoded by all or part of these sequences, in the development of vaccines and diagnostic assays for HCV and in the development of screening assays for the identification of antiviral agents for HCV.

Background Of Invention

Hepatitis C virus (HCV) has a positive-sense single-strand RNA genome and is a member of the virus family *Flaviviridae* (Choo et al., 1991; Rice, 1996). As for all positive-stranded RNA viruses, the genome of HCV functions as mRNA from which all viral proteins necessary for propagation are translated.

The viral genome of HCV is approximately 9600 nucleotides (nts) and consists of a highly conserved 5′ untranslated region (UTR), a single long open reading frame (ORF) of approximately 9,000 nts and a complex 3′ UTR. The 5′ UTR contains an internal ribosomal entry site (Tsukiyama-Kohara et al., 1992; Honda et al., 1996). The 3′ UTR consists of a short variable region, a polypyrimidine tract of variable length and, at the 3′ end, a highly conserved region of approximately 100 nts (Kolykhalov et al., 1996; Tanaka et al., 1995; Tanaka et

al., 1996; Yamada et al., 1996). The last 46 nucleotides of this conserved region were predicted to form a stable stem-loop structure thought to be critical for viral replication (Blight and Rice, 1997; Ito and Lai, 1997; Tsuchihara et al., 1997). The ORF encodes a large polypeptide precursor that is cleaved into at least 10 proteins by host and viral proteinases (Rice, 1996). The predicted envelope proteins contain several conserved N-linked glycosylation sites and cysteine residues (Okamoto et al., 1992a). The NS3 gene encodes a serine protease and an RNA helicase and the NS5B gene encodes an RNA-dependent RNA polymerase.

Globally, six major HCV genotypes (genotypes 1-6) and multiple subtypes (a, b, c, etc.) have been identified (Bukh et al., 1993; Simmonds et al., 1993). The most divergent HCV isolates differ from each other by more than 30% over the entire genome (Okamoto et al., 1992a) and HCV circulates in an infected individual as a quasispecies of closely related genomes (Bukh et al., 1995; Farci et al., 1997).

At present, more than 80% of individuals infected with HCV become chronically infected and these chronically infected individuals have a relatively high risk of developing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Hoofnagle, 1997). In the U.S., HCV genotypes 1a and 1b constitute the majority of infections while in many other areas, especially in Europe and Japan, genotype 1b predominates.

The only effective therapy for chronic hepatitis C, interferon (IFN), induces a sustained response in less than 25% of treated patients (Fried and Hoofnagle, 1995). Consequently, HCV is currently the most common cause of end stage liver failure and the reason for about 30% of liver transplants performed in the U.S. (Hoofnagle, 1997). In addition, a number of recent studies suggested that the severity of liver disease and the outcome of therapy may

be genotype-dependent (reviewed in Bukh et al., 1997). In particular, these studies suggested that infection with HCV genotype 1b was associated with more severe liver disease (Brechot, 1997) and a poorer response to IFN therapy (Fried and Hoofnagle, 1995). As a result of the inability to develop a universally effective therapy against HCV infection, it is estimated that there are still more than 25,000 new infections yearly in the U.S. (Alter 1997) Moreover, since there is no vaccine for HCV, HCV remains a serious public health problem.

However, despite the intense interest in the development of vaccines and therapies for HCV, progress has been hindered by the absence of a useful cell culture system and the lack of any small animal model for laboratory study. For example, while replication of HCV in several cell lines has been reported, such observations have turned out not to be highly reproducible. In addition, the chimpanzee is the only animal model, other than man, for this disease. Consequently, HCV has been able to be studied only by using clinical materials obtained from patients or experimentally infected chimpanzees (an animal model whose availability is very limited).

However, several researchers have recently reported the construction of infectious cDNA clones of HCV, the identification of which would permit a more effective search for susceptible cell lines and facilitate molecular analysis of the viral genes and their function. For example, Dash et al., (1997) and Yoo et al., (1995) reported that RNA transcripts from cDNA clones of HCV-1 (genotype 1a) and HCV-N (genotype 1b), respectively, resulted in viral replication after transfection into human hepatoma cell lines. Unfortunately, the viability of these clones was not tested in vivo and concerns were raised about the infectivity of these cDNA clones in vitro (Fausto, 1997). In addition, both clones did not contain

the terminal 98 conserved nucleotides at the very 3' end of the UTR.

Kolykhalov et al., (1997) and Yanagi et al. (1997) reported the derivation from HCV strain H77 (which is genotype 1a) of cDNA clones of HCV that are infectious for chimpanzees. However, while these infectious clones will aid in studying HCV replication and pathogenesis and will provide an important tool for development of in vitro replication and propagation systems, it is important to have infectious clones of more than one genotype given the extensive genetic heterogeneity of HCV and the potential impact of such heterogeneity on the development of effective therapies and vaccines for HCV.

Summary Of The Invention

The present invention relates to nucleic acid sequences which comprise the genome of infectious hepatitis C viruses and in particular, nucleic acid sequences which comprise the genome of infectious hepatitis C viruses of genotype 1a and 1b strains. It is therefore an object of the invention to provide nucleic acid sequences which encode infectious hepatitis C viruses. Such nucleic acid sequences are referred to throughout the application as "infectious nucleic acid sequences".

For the purposes of this application, nucleic acid sequence refers to RNA, DNA, cDNA or any variant thereof capable of directing host organism synthesis of a hepatitis C virus polypeptide. It is understood that nucleic acid sequence encompasses nucleic acid sequences, which due to degeneracy, encode the same polypeptide sequence as the nucleic acid sequences described herein.

The invention also relates to the use of the infectious nucleic acid sequences to produce chimeric genomes consisting of portions of the open reading frames of infectious nucleic acid sequences of other genotypes (including, but not limited to, genotypes 1, 2, 3, 4, 5

and 6) and subtypes (including, but not limited to, subtypes 1a, 1b, 2a, 2b, 2c, 3a 4a-4f, 5a and 6a) of HCV. For example infectious nucleic acid sequence of the 1a and 1b strains H77 and HC-J4, respectively, described herein can be used to produce chimeras with sequences from the genomes of other strains of HCV from different genotypes or subtypes. Nucleic acid sequences which comprise sequence from the open-reading frames of 2 or more HCV genotypes or subtypes are designated "chimeric nucleic acid sequences".

The invention further relates to mutations of the infectious nucleic acid sequences of the invention where mutation includes, but is not limited to, point mutations, deletions and insertions. In one embodiment, a gene or fragment thereof can be deleted to determine the effect of the deleted gene or genes on the properties of the encoded virus such as its virulence and its ability to replicate. In an alternative embodiment, a mutation may be introduced into the infectious nucleic acid sequences to examine the effect of the mutation on the properties of the virus in the host cell.

The invention also relates to the introduction of mutations or deletions into the infectious nucleic acid sequences in order to produce an attenuated hepatitis C virus suitable for vaccine development.

The invention further relates to the use of the infectious nucleic acid sequences to produce attenuated viruses via passage in vitro or in vivo of the viruses produced by transfection of a host cell with the infectious nucleic acid sequence.

The present invention also relates to the use of the nucleic acid sequences of the invention or fragments thereof in the production of polypeptides where "nucleic acid sequences of the invention" refers to infectious nucleic acid sequences, mutations of infectious nucleic acid sequences, chimeric nucleic acid sequences and

sequences which comprise the genome of attenuated viruses produced from the infectious nucleic acid sequences of the invention. The polypeptides of the invention, especially structural polypeptides, can serve as immunogens in the development of vaccines or as antigens in the development of diagnostic assays for detecting the presence of HCV in biological samples.

The invention therefore also relates to vaccines for use in immunizing mammals especially humans against hepatitis C. In one embodiment, the vaccine comprises one or more polypeptides made from a nucleic acid sequence of the invention or fragment thereof. In a second embodiment, the vaccine comprises a hepatitis C virus produced by transfection of host cells with the nucleic acid sequences of the invention.

The present invention therefore relates to methods for preventing hepatitis C in a mammal. In one embodiment the method comprises administering to a mammal a polypeptide or polypeptides encoded by a nucleic acid sequence of the invention in an amount effective to induce protective immunity to hepatitis C. In another embodiment, the method of prevention comprises administering to a mammal a hepatitis C virus of the invention in an amount effective to induce protective immunity against hepatitis C.

In yet another embodiment, the method of protection comprises administering to a mammal a nucleic acid sequence of the invention or a fragment thereof in an amount effective to induce protective immunity against hepatitis C.

The invention also relates to hepatitis C viruses produced by host cells transfected with the nucleic acid sequences of the present invention.

The invention therefore also provides pharmaceutical compositions comprising the nucleic acid sequences of the invention and/or their encoded hepatitis

C viruses. The invention further provides pharmaceutical compositions comprising polypeptides encoded by the nucleic acid sequences of the invention or fragments thereof. The pharmaceutical compositions of the invention may be used prophylactically or therapeutically.

The invention also relates to antibodies to the hepatitis C viruses of the invention or their encoded polypeptides and to pharmaceutical compositions comprising these antibodies.

The present invention further relates to polypeptides encoded by the nucleic acid sequences of the invention fragments thereof. In one embodiment, said polypeptide or polypeptides are fully or partially purified from hepatitis C virus produced by cells transfected with nucleic acid sequence of the invention. In another embodiment, the polypeptide or polypeptides are produced recombinantly from a fragment of the nucleic acid sequences of the invention. In yet another embodiment, the polypeptides are chemically synthesized.

The invention also relates to the use of the nucleic acid sequences of the invention to identify cell lines capable of supporting the replication of HCV in vitro.

The invention further relates to the use of the nucleic acid sequences of the invention or their encoded proteases (e.g. NS3 protease) to develop screening assays to identify antiviral agents for HCV.

Brief Description Of Figures

Figure **1** shows a strategy for constructing full-length cDNA clones of HCV strain H77. The long PCR products amplified with H1 and H9417R primers were cloned directly into pGEM-9zf(-) after digestion with Not I and Xba I (pH21, and pH50,). Next, the 3' UTR was cloned into both pH21, and pH50, after digestion with Afl II and Xba I (pH21 and pH50). pH21 was tested for infectivity in a chimpanzee. To improve the efficiency of cloning, we

constructed a cassette vector with consensus 5' and 3' termini of H77. This cassette vector (pCV) was obtained by cutting out the BamHI fragment (nts 1358- 7530 of the H77 genome) from pH50, followed by religation. Finally, the long PCR products of H77 amplified with primers H1 and H9417R (H product) or primers A1 and H9417R (A product) were cloned into pCV after digestion with Age I and Afl II or with Pin AI and Bfr I. The latter procedure yielded multiple complete cDNA clones of strain H77 of HCV.

Figure **2** shows the results of gel electrophoresis of long RT-PCR amplicons of the entire ORF of H77 and the transcription mixture of the infectious clone of H77. The complete ORF was amplified by long RT-PCR with the primers H1 or A1 and H9417R from 10^5 GE of H77. A total of 10 μg of the consensus chimeric clone (pCV-H77C) linearized with Xba I was transcribed in a 100 μl reaction with T7 RNA polymerase. Five μl of the transcription mixture was analyzed by gel electrophoresis and the remainder of the mixture was injected into a chimpanzee. Lane 1, molecular weight marker ; lane 2, products amplified with primers H1 and H9417R; lane 3, products amplified with primers A1 and H9417R; lane 4, transcription mixture containing the RNA transcripts and linearized clone pCV-H77C (12.5 kb).

Figure **3** is a diagram of the genome organization of HCV strain H77 and the genetic heterogeneity of individual full-length clones compared with the consensus sequence of H77. Solid lines represent aa changes. Dashed lines represent silent mutations. A * in pH21 represents a point mutation at nt 58 in the 5' UTR. In the ORF, the consensus chimeric clone pCV-H77C had 11 nt differences [at positions 1625 (C→T), 2709 (T→C), 3380 (A→G), 3710 (C→T), 3914 (G→A), 4463 (T→C), 5058 (C→T), 5834 (C→T), 6734 (T→C), 7154 (C→T), and 7202 (T→C)] and one aa change (F → L at aa 790) compared with the consensus sequence of H77. This clone was infectious.

Clone pH21 and pCV-H11 had 19 nts (7 aa) and 64 nts (21 aa) differences respectively, compared with the consensus sequence of H77. These two clones were not infectious. A single point mutation in the 3' UTR at nucleotide 9406 (G→A) introduced to create an *Afl* II cleavage site is not shown.

Figures **4A-4F** show the complete nucleotide sequence of a H77C clone produced according to the present invention and Figures **4G-4H** show the amino acid sequence encoded by the H77C clone.

Figure **5** shows an agarose gel of long RT-PCR amplicons and transcription mixtures. Lanes 1 and 4: Molecular weight marker (Lambda/*Hin*dIII digest). Lanes 2 and 3: RT-PCR amplicons of the entire ORF of HC-J4. Lane 5: pCV-H77C transcription control (Yanagi et al., 1997). Lanes 6, 7, and 8: 1/40 of each transcription mixture of pCV-J4L2S, pCV-J4L4S and pCV-J4L6S, respectively, which was injected into the chimpanzee.

Figure **6** shows the strategy utilized for the construction of full-length cDNA clones of HCV strain HC-J4. The long PCR products were cloned as two separate fragments (L and S) into a cassette vector (pCV) with fixed 5' and 3' termini of HCV (Yanagi et al., 1997). Full-length cDNA clones of HC-J4 were obtained by inserting the L fragment from three pCV-J4L clones into three identical pCV-J4S9 clones after digestion with *Pin*AI (isoschizomer of *Age*I) and *Bfr*I (isoschizomer of *Afl*II).

Figure **7** shows amino acid positions with a quasispecies of HC-J4 in the acute phase plasma pool obtained from an experimentally infected chimpanzee. Cons-p9: consensus amino acid sequence deduced from analysis of nine L fragments and nine S fragments (see Fig. **6**). Cons-D: consensus sequence derived from direct sequencing of the PCR product. A, B, C: groups of similar viral species. Dot: amino acid identical to that in Cons-

p9. Capital letter: amino acid different from that in Cons-p9. Cons-F: composite consensus amino acid sequence combining Cons-p9 and Cons-D. Boxed amino acid: different from that in Cons-F. Shaded amino acid: different from that in all species A sequences. An *: defective ORF due to a nucleotide deletion (clone L1, aa 1097) or insertion (clone L7, aa 2770). Diagonal lines: fragments used to construct the infectious clone.

Figure **8** shows comparisons (percent difference) of nucleotide (nts. 156 - 8935) and predicted amino acid sequences (aa 1 - 2864) of L clones (species A, B, and C, this study), HC-J4/91 (Okamoto et al., 1992b) and HC-J4/83 (Okamoto et al., 1992b). Differences among species A sequences and among species B sequences are shaded.

Figure **9** shows UPGMA ("unweighted pair group method with arithmetic mean") trees of HC-J4/91 (Okamoto et al., 1992b), HC-J4/83 (Okamoto et al., 1992b), two prototype strains of genotype 1b (HCV-J, Kato et al., 1990; HCV-BK, Takamizawa et al., 1991), and L clones (this study).

Figure **10** shows the alignment of the HVR1 and HVR2 amino acid sequences of the E2 sequences of nine L clones of HC-J4 (species A, B, and C) obtained from an early acute phase plasma pool of an experimentally infected chimpanzee compared with the sequences of eight clones (HC-J4/91-20 through HC-J4/91-27, Okamoto et al., 1992b) derived from the inoculum. Dot: an amino acid identical to that in the top line. Capital letters: amino acid different from that in the top line.

Figure **11** shows the alignment of the 5′ UTR and the 3′ UTR sequences of infectious clones of genotype 1a (pCV-H77C) and 1b (pCV-J4L6S). Top line: consensus sequence of the indicated strain. Dot: identity with consensus sequence. Capital letter: different from the consensus sequence. Dash: deletion. Underlined: *Pin*AI and *Bfr*I cleavage site. Numbering corresponds to the HCV

sequence of pCV-J4L6S.

Figure **12** shows a comparison of individual full-length cDNA clones of the ORF of HCV strain HC-J4 with the consensus sequence (see Fig. **7**). Solid lines: amino acid changes. Dashed lines: silent mutations. Clone pCV-J4L6S was infectious in vivo whereas clones pCV-J4L2S and pCV-J4L4S were not.

Figure **13** shows biochemical (ALT levels) and PCR analyses of a chimpanzee following percutaneous intrahepatic transfection with RNA transcripts of the infectious clone of pCV-J4L2S, pCV-J4L4S and pCV-J4L6S. The ALT serum enzyme levels were measured in units per liter (u/l). For the PCR analysis, "HCV RNA" represented by an open rectangle indicates a serum sample that was negative for HCV after nested PCR; "HCV RNA" represented by a closed rectangle indicates that the serum sample was positive for HCV and HCV GE titer on the right-hand y-axis represents genome equivalents.

Figures **14A-14F** show the nucleotide sequence of the infectious clone of genotype 1b strain HC-J4 and Figures **14G-14H** show the amino acid sequence encoded by the HC-J4 clone.

Figure **15** shows the strategy for constructing a chimeric HCV clone designated pH77CV-J4 which contains the nonstructural region of the infectious clone of genotype 1a strain H77 and the structural region of the infectious clone of genotype 1b strain HC-J4.

Figures **16A-16F** show the nucleotide sequence of the chimeric 1a/1b clone pH77CV-J4 of Figure **15** and Figures **16G-16H** show the amino acid sequence encoded by the chimeric 1a/1b clone.

Figures **17A and 17B** show the sequence of the 3' untranslated region remaining in various 3' deletion mutants of the 1a infectious clone pCV-H77C and the strategy utilized in constructing each 3' deletion mutant (Figures **17C-17G**).

Of the seven deletion mutants shown, two (pCV-H77C(-98X) and (-42X)) have been constructed and tested for infectivity in chimpanzees (see Figures **17A** and **17C**) and the other six are to be constructed and tested for infectivity as described in Figures **17D-17G**.

Figures **18A and 18B** show biochemical (ALT levels), PCR (HCV RNA and HCV GE titer), serological (anti-HCV) and histopathological (Fig. **18B** only) analyses of chimpanzees 1494 (Fig. **18A**) and 1530 (Fig. **18B**) following transfection with the infectious cDNA clone pCV-H77C.

The ALT serum enzyme levels were measured in units per ml (u/l). For the PCR analysis, "HCV RNA" represented by an open rectangle indicates a serum sample that was negative for HCV after nested PCR; "HCV RNA" represented by a closed rectangle indicates that the serum sample was positive for HCV; and HCV GE titer on the right-hand y-axis represents genome equivalents.

The bar marked "anti-HCV" indicates samples that were positive for anti-HCV antibodies as determined by commercial assays. The histopathology scores in Figure 18B correspond to no histopathology (O), mild hepatitis (⊖) and moderate to severe hepatitis (●).

DESCRIPTION OF THE INVENTION

The present invention relates to nucleic acid sequences which comprise the genome of an infectious hepatitis C virus. More specifically, the invention relates to nucleic acid sequences which encode infectious hepatitis C viruses of genotype 1a and 1b strains. In one embodiment, the infectious nucleic acid sequence of the invention has the sequence shown in Figures **4A-4F** of this application. In another embodiment, the infectious nucleic acid sequence has the sequence shown in Figures **14A-14F** and is contained in a plasmid construct deposited with the American Type Culture Collection (ATCC) on January 26, 1998 and having ATCC accession number ____.

The invention also relates to "chimeric nucleic acid sequences" where the chimeric nucleic acid sequences consist of open-reading frame sequences taken from infectious nucleic acid sequences of hepatitis C viruses of different genotypes or subtypes.

In one embodiment, the chimeric nucleic acid sequence consists of sequence from the genome of an HCV strain belonging to one genotype or subtype which encodes structural polypeptides and sequence of an HCV strain belonging to another genotype strain or subtype which encodes nonstructural polypeptides. Such chimeras can be produced by standard techniques of restriction digestion, PCR amplification and subcloning known to those of ordinary skill in the art.

In a preferred embodiment, the sequence encoding nonstructural polypeptides is from an infectious nucleic acid sequence encoding a genotype 1a strain where the construction of a chimeric 1a/1b nucleic acid sequence is described in Example **9** and the chimeric 1a/1b nucleic acid sequence is shown in Figures **16A-16F**. It is believed that the construction of such chimeric nucleic acid sequences will be of importance in studying the growth and virulence properties of hepatitis C virus and in the production of hepatitis C viruses suitable to confer protection against multiple genotypes of HCV. For example, one might produce a "multivalent" vaccine by putting epitopes from several genotypes or subtypes into one clone. Alternatively one might replace just a single gene from an infectious sequence with the corresponding gene from the genomic sequence of a strain from another genotype or subtype or create a chimeric gene which contains portions of a gene from two genotypes or subtypes. Examples of genes which could be replaced or which could be made chimeric, include, but are not limited to, the E1, E2 and NS4 genes.

The invention further relates to mutations of the infectious nucleic acid sequences where "mutations"

includes, but is not limited to, point mutations, deletions and insertions. Of course, one of ordinary skill in the art would recognize that the size of the insertions would be limited by the ability of the resultant nucleic acid sequence to be properly packaged within the virion. Such mutation could be produced by techniques known to those of skill in the art such as site-directed mutagenesis, fusion PCR, and restriction digestion followed by religation.

In one embodiment, mutagenesis might be undertaken to determine sequences that are important for viral properties such as replication or virulence. For example, one may introduce a mutation into the infectious nucleic acid sequence which eliminates the cleavage site between the NS4A and NS4B polypeptides to examine the effects on viral replication and processing of the polypeptide. Alternatively, one or more of the 3 amino acids encoded by the infectious 1b nucleic acid sequence shown in Figures **14A-14F** which differ from the HC-J4 consensus sequence may be back mutated to the corresponding amino acid in the HC-J4 consensus sequence to determine the importance of these three amino acid changes to infectivity or virulence. In yet another embodiment, one or more of the amino acids from the noninfectious 1b clones pCV-J4L2S and pCV-J4L4S which differ from the consensus sequence may be introduced into the infectious 1b sequence shown in Figures **14A-14F**.

In yet another example, one may delete all or part of a gene or of the 5' or 3' nontranslated region contained in an infectious nucleic acid sequence and then transfect a host cell (animal or cell culture) with the mutated sequence and measure viral replication in the host by methods known in the art such as RT-PCR. Preferred genes include, but are not limited to, the P7, NS4B and NS5A genes. Of course, those of ordinary skill in the art will understand that deletion of part of a gene,

preferably the central portion of the gene, may be preferable to deletion of the entire gene in order to conserve the cleavage site boundaries which exist between proteins in the HCV polyprotein and which are necessary for proper processing of the polyprotein.

In the alternative, if the transfection is into a host animal such as a chimpanzee, one can monitor the virulence phenotype of the virus produced by transfection of the mutated infectious nucleic acid sequence by methods known in the art such as measurement of liver enzyme levels (alanine aminotransferase (ALT) or isocitrate dehydrogenase (ICD)) or by histopathology of liver biopsies. Thus, mutations of the infectious nucleic acid sequences may be useful in the production of attenuated HCV strains suitable for vaccine use.

The invention also relates to the use of the infectious nucleic acid sequences of the present invention to produce attenuated viral strains via passage in vitro or in vivo of the virus produced by transfection with the infectious nucleic acid sequences.

The present invention therefore relates to the use of the nucleic acid sequences of the invention to identify cell lines capable of supporting the replication of HCV.

In particular, it is contemplated that the mutations of the infectious nucleic acid sequences of the invention and the production of chimeric sequences as discussed above may be useful in identifying sequences critical for cell culture adaptation of HCV and hence, may be useful in identifying cell lines capable of supporting HCV replication.

Transfection of tissue culture cells with the nucleic acid sequences of the invention may be done by methods of transfection known in the art such as electroporation, precipitation with DEAE-Dextran or calcium phosphate or liposomes.

In one such embodiment, the method comprises the growing of animal cells, especially human cells, in vitro and transfecting the cells with the nucleic acid of the invention, then determining if the cells show indicia of HCV infection. Such indicia include the detection of viral antigens in the cell, for example, by immunofluorescent procedures well known in the art; the detection of viral polypeptides by Western blotting using antibodies specific therefor; and the detection of newly transcribed viral RNA within the cells via methods such as RT-PCR. The presence of live, infectious virus particles following such tests may also be shown by injection of cell culture medium or cell lysates into healthy, susceptible animals, with subsequent exhibition of the symptoms of HCV infection.

Suitable cells or cell lines for culturing HCV include, but are not limited to, lymphocyte and hepatocyte cell lines known in the art.

Alternatively, primary hepatocytes can be cultured, and then infected with HCV; or, the hepatocyte cultures could be derived from the livers of infected chimpanzees. In addition, various immortalization methods known to those of ordinary skill in the art can be used to obtain cell-lines derived from hepatocyte cultures. For example, primary hepatocyte cultures may be fused to a variety of cells to maintain stability.

The present invention further relates to the in vitro and in vivo production of hepatitis C viruses from the nucleic acid sequences of the invention.

In one embodiment, the sequences of the invention can be inserted into an expression vector that functions in eukaryotic cells. Eukaryotic expression vectors suitable for producing high efficiency gene transfer in vivo are well known to those of ordinary skill in the art and include, but are not limited to, plasmids, vaccinia viruses, retroviruses, adenoviruses and adeno-

associated viruses.

In another embodiment, the sequences contained in the recombinant expression vector can be transcribed _in vitro_ by methods known to those of ordinary skill in the art in order to produce RNA transcripts which encode the hepatitis C viruses of the invention. The hepatitis C viruses of the invention may then be produced by transfecting cells by methods known to those of ordinary skill in the art with either the _in vitro_ transcription mixture containing the RNA transcripts (see Example 4) or with the recombinant expression vectors containing the nucleic acid sequences described herein.

The present invention also relates to the construction of cassette vectors useful in the cloning of viral genomes wherein said vectors comprise a nucleic acid sequence to be cloned, and said vector reading in the correct phase for the expression of the viral nucleic acid to be cloned. Such a cassette vector will, of course, also possess a promoter sequence, advantageously placed upstream of the sequence to be expressed. Cassette vectors according to the present invention are constructed according to the procedure described in Figure 1, for example, starting with plasmid pCV. Of course, the DNA to be inserted into said cassette vector can be derived from any virus, advantageously from HCV, and most advantageously from the H77 strain of HCV. The nucleic acid to be inserted according to the present invention can, for example, contain one or more open reading frames of the virus, for example, HCV. The cassette vectors of the present invention may also contain, optionally, one or more expressible marker genes for expression as an indication of successful transfection and expression of the nucleic acid sequences of the vector. To insure expression, the cassette vectors of the present invention will contain a promoter sequence for binding of the appropriate cellular RNA polymerase, which will depend on

the cell into which the vector has been introduced. For example, if the host cell is a bacterial cell, then said promoter will be a bacterial promoter sequence to which the bacterial RNA polymerases will bind.

The hepatitis C viruses produced from the sequences of the invention may be purified or partially purified from the transfected cells by methods known to those of ordinary skill in the art. In a preferred embodiment, the viruses are partially purified prior to their use as immunogens in the pharmaceutical compositions and vaccines of the present invention.

The present invention therefore relates to the use of the hepatitis C viruses produced from the nucleic acid sequences of the invention as immunogens in live or killed (e.g., formalin inactivated) vaccines to prevent hepatitis C in a mammal.

In an alternative embodiment, the immunogen of the present invention may be an infectious nucleic acid sequence, a chimeric nucleic acid sequence, or a mutated infectious nucleic acid sequence which encodes a hepatitis C virus. Where the sequence is a cDNA sequence, the cDNAs and their RNA transcripts may be used to transfect a mammal by direct injection into the liver tissue of the mammal as described in the Examples.

Alternatively, direct gene transfer may be accomplished via administration of a eukaryotic expression vector containing a nucleic acid sequence of the invention.

In yet another embodiment, the immunogen may be a polypeptide encoded by the nucleic acid sequences of the invention. The present invention therefore also relates to polypeptides produced from the nucleic acid sequences of the invention or fragments thereof. In one embodiment, polypeptides of the present invention can be recombinantly produced by synthesis from the nucleic acid sequences of the invention or isolated fragments thereof, and purified,

or partially purified, from transfected cells using methods already known in the art. In an alternative embodiment, the polypeptides may be purified or partially purified from viral particles produced via transfection of a host cell with the nucleic acid sequences of the invention. Such polypeptides might, for example, include either capsid or envelope polypeptides prepared from the sequences of the present invention.

When used as immunogens, the nucleic acid sequences of the invention, or the polypeptides or viruses produced therefrom, are preferably partially purified prior to use as immunogens in pharmaceutical compositions and vaccines of the present invention. When used as a vaccine, the sequences and the polypeptide and virus products thereof, can be administered alone or in a suitable diluent, including, but not limited to, water, saline, or some type of buffered medium. The vaccine according to the present invention may be administered to an animal, especially a mammal, and most especially a human, by a variety of routes, including, but not limited to, intradermally, intramuscularly, subcutaneously, or in any combination thereof.

Suitable amounts of material to administer for prophylactic and therapeutic purposes will vary depending on the route selected and the immunogen (nucleic acid, virus, polypeptide) administered. One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can be readily determined without undue experimentation. The vaccines of the present invention may be administered once or periodically until a suitable titer of anti-HCV antibodies appear in the blood. For an immunogen consisting of a nucleic acid sequence, a suitable amount of nucleic acid sequence to be used for prophylactic purposes might be expected to fall in the range of from about 100 μg to about 5 mg and most preferably in the range of from about

500 μg to about 2mg. For a polypeptide, a suitable amount to use for prophylactic purposes is preferably 100 ng to 100 μg and for a virus 10^2 to 10^6 infectious doses. Such administration will, of course, occur prior to any sign of HCV infection.

A vaccine of the present invention may be employed in such forms as capsules, liquid solutions, suspensions or elixirs for oral administration, or sterile liquid forms such as solutions or suspensions. Any inert carrier is preferably used, such as saline or phosphate-buffered saline, or any such carrier in which the HCV of the present invention can be suitably suspended. The vaccines may be in the form of single dose preparations or in multi-dose flasks which can be utilized for mass-vaccination programs of both animals and humans. For purposes of using the vaccines of the present invention reference is made to Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., Osol (Ed.) (1980); and New Trends and Developments in Vaccines, Voller et al. (Eds.), University Park Press, Baltimore, Md. (1978), both of which provide much useful information for preparing and using vaccines. Of course, the polypeptides of the present invention, when used as vaccines, can include, as part of the composition or emulsion, a suitable adjuvant, such as alum (or aluminum hydroxide) when humans are to be vaccinated, to further stimulate production of antibodies by immune cells. When nucleic acids or viruses are used for vaccination purposes, other specific adjuvants such as CpG motifs (Krieg, A.K. et al.(1995) and (1996)), may prove useful.

When the nucleic acids, viruses and polypeptides of the present invention are used as vaccines or inocula, they will normally exist as physically discrete units suitable as a unitary dosage for animals, especially mammals, and most especially humans, wherein each unit will contain a predetermined quantity of active material

calculated to produce the desired immunogenic effect in association with the required diluent. The dose of said vaccine or inoculum according to the present invention is administered at least once. In order to increase the antibody level, a second or booster dose may be administered at some time after the initial dose. The need for, and timing of, such booster dose will, of course, be determined within the sound judgment of the administrator of such vaccine or inoculum and according to sound principles well known in the art. For example, such booster dose could reasonably be expected to be advantageous at some time between about 2 weeks to about 6 months following the initial vaccination. Subsequent doses may be administered as indicated.

The nucleic acid sequences, viruses and polypeptides of the present invention can also be administered for purposes of therapy, where a mammal, especially a primate, and most especially a human, is already infected, as shown by well known diagnostic measures. When the nucleic acid sequences, viruses or polypeptides of the present invention are used for such therapeutic purposes, much of the same criteria will apply as when it is used as a vaccine, except that inoculation will occur post-infection. Thus, when the nucleic acid sequences, viruses or polypeptides of the present invention are used as therapeutic agents in the treatment of infection, the therapeutic agent comprises a pharmaceutical composition containing a sufficient amount of said nucleic acid sequences, viruses or polypeptides so as to elicit a therapeutically effective response in the organism to be treated. Of course, the amount of pharmaceutical composition to be administered will, as for vaccines, vary depending on the immunogen contained therein (nucleic acid, polypeptide, virus) and on the route of administration.

The therapeutic agent according to the present

invention can thus be administered by, subcutaneous, intramuscular or intradermal routes. One skilled in the art will certainly appreciate that the amounts to be administered for any particular treatment protocol can be readily determined without undue experimentation. Of course, the actual amounts will vary depending on the route of administration as well as the sex, age, and clinical status of the subject which, in the case of human patients, is to be determined with the sound judgment of the clinician.

The therapeutic agent of the present invention can be employed in such forms as capsules, liquid solutions, suspensions or elixirs, or sterile liquid forms such as solutions or suspensions. Any inert carrier is preferably used, such as saline, phosphate-buffered saline, or any such carrier in which the HCV of the present invention can be suitably suspended. The therapeutic agents may be in the form of single dose preparations or in the multi-dose flasks which can be utilized for mass-treatment programs of both animals and humans. Of course, when the nucleic acid sequences, viruses or polypeptides of the present invention are used as therapeutic agents they may be administered as a single dose or as a series of doses, depending on the situation as determined by the person conducting the treatment.

The nucleic acids, polypeptides and viruses of the present invention can also be utilized in the production of antibodies against HCV. The term "antibody" is herein used to refer to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules. Examples of antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and portions of an immunoglobulin molecule, including those portions known in the art as Fab, $F(ab')_2$ and F(v) as well as chimeric antibody molecules.

Thus, the polypeptides, viruses and nucleic acid sequences of the present invention can be used in the generation of antibodies that immunoreact (i.e., specific binding between an antigenic determinant-containing molecule and a molecule containing an antibody combining site such as a whole antibody molecule or an active portion thereof) with antigenic determinants on the surface of hepatitis C virus particles.

The present invention therefore also relates to antibodies produced following immunization with the nucleic acid sequences, viruses or polypeptides of the present invention. These antibodies are typically produced by immunizing a mammal with an immunogen or vaccine to induce antibody molecules having immunospecificity for polypeptides or viruses produced in response to infection with the nucleic acid sequences of the present invention. When used in generating such antibodies, the nucleic acid sequences, viruses, or polypeptides of the present invention may be linked to some type of carrier molecule. The resulting antibody molecules are then collected from said mammal. Antibodies produced according to the present invention have the unique advantage of being generated in response to authentic, functional polypeptides produced according to the actual cloned HCV genome.

The antibody molecules of the present invention may be polyclonal or monoclonal. Monoclonal antibodies are readily produced by methods well known in the art. Portions of immunoglobin molecules, such as Fabs, as well as chimeric antibodies, may also be produced by methods well known to those of ordinary skill in the art of generating such antibodies.

The antibodies according to the present invention may also be contained in blood plasma, serum, hybridoma supernatants, and the like. Alternatively, the antibody of the present invention is isolated to the

extent desired by well known techniques such as, for example, using DEAE Sephadex. The antibodies produced according to the present invention may be further purified so as to obtain specific classes or subclasses of antibody such as IgM, IgG, IgA, and the like. Antibodies of the IgG class are preferred for purposes of passive protection.

The antibodies of the present invention are useful in the prevention and treatment of diseases caused by hepatitis C virus in animals, especially mammals, and most especially humans.

In providing the antibodies of the present invention to a recipient mammal, preferably a human, the dosage of administered antibodies will vary depending on such factors as the mammal's age, weight, height, sex, general medical condition, previous medical history, and the like.

In general, it will be advantageous to provide the recipient mammal with a dosage of antibodies in the range of from about 1 mg/kg body weight to about 10 mg/kg body weight of the mammal, although a lower or higher dose may be administered if found desirable. Such antibodies will normally be administered by intravenous or intramuscular route as an inoculum. The antibodies of the present invention are intended to be provided to the recipient subject in an amount sufficient to prevent, lessen or attenuate the severity, extent or duration of any existing infection.

The antibodies prepared by use of the nucleic acid sequences, viruses or polypeptides of the present invention are also highly useful for diagnostic purposes. For example, the antibodies can be used as in vitro diagnostic agents to test for the presence of HCV in biological samples taken from animals, especially humans. Such assays include, but are not limited to, radioimmunoassays, EIA, fluorescence, Western blot

analysis and ELISAs. In one such embodiment, the biological sample is contacted with antibodies of the present invention and a labeled second antibody is used to detect the presence of HCV to which the antibodies are bound.

Such assays may be, for example, a direct protocol (where the labeled first antibody is immunoreactive with the antigen, such as, for example, a polypeptide on the surface of the virus), an indirect protocol (where a labeled second antibody is reactive with the first antibody), a competitive protocol (such as would involve the addition of a labeled antigen), or a sandwich protocol (where both labeled and unlabeled antibody are used), as well as other protocols well known and described in the art.

In one embodiment, an immunoassay method would utilize an antibody specific for HCV envelope determinants and would further comprise the steps of contacting a biological sample with the HCV-specific antibody and then detecting the presence of HCV material in the test sample using one of the types of assay protocols as described above. Polypeptides and antibodies produced according to the present invention may also be supplied in the form of a kit, either present in vials as purified material, or present in compositions and suspended in suitable diluents as previously described.

In a preferred embodiment, such a diagnostic test kit for detection of HCV antigens in a test sample comprises in combination a series of containers, each container a reagent needed for such assay. Thus, one such container would contain a specific amount of HCV-specific antibody as already described, a second container would contain a diluent for suspension of the sample to be tested, a third container would contain a positive control and an additional container would contain a negative control. An additional container could contain a blank.

For all prophylactic, therapeutic and diagnostic uses, the antibodies of the invention and other reagents, plus appropriate devices and accessories, may be provided in the form of a kit so as to facilitate ready availability and ease of use.

The present invention also relates to the use of nucleic acid sequences and polypeptides of the present invention to screen potential antiviral agents for antiviral activity against HCV. Such screening methods are known by those of skill in the art. Generally, the antiviral agents are tested at a variety of concentrations, for their effect on preventing viral replication in cell culture systems which support viral replication, and then for an inhibition of infectivity or of viral pathogenicity (and a low level of toxicity) in an animal model system.

In one embodiment, animal cells (especially human cells) transfected with the nucleic acid sequences of the invention are cultured in vitro and the cells are treated with a candidate antiviral agent (a chemical, peptide etc.) for antiviral activity by adding the candidate agent to the medium. The treated cells are then exposed, possibly under transfecting or fusing conditions known in the art, to the nucleic acid sequences of the present invention. A sufficient period of time would then be allowed to pass for infection to occur, following which the presence or absence of viral replication would be determined versus untreated control cells by methods known to those of ordinary skill in the art. Such methods include, but are not limited to, the detection of viral antigens in the cell, for example, by immunofluorescent procedures well known in the art; the detection of viral polypeptides by Western blotting using antibodies specific therefor; the detection of newly transcribed viral RNA within the cells by RT-PCR; and the detection of the presence of live, infectious virus particles by injection

of cell culture medium or cell lysates into healthy, susceptible animals, with subsequent exhibition of the symptoms of HCV infection. A comparison of results obtained for control cells (treated only with nucleic acid sequence) with those obtained for treated cells (nucleic acid sequence and antiviral agent) would indicate, the degree, if any, of antiviral activity of the candidate antiviral agent. Of course, one of ordinary skill in the art would readily understand that such cells can be treated with the candidate antiviral agent either before or after exposure to the nucleic acid sequence of the present invention so as to determine what stage, or stages, of viral infection and replication said agent is effective against.

In an alternative embodiment, a protease such as NS3 protease produced from a nucleic acid sequence of the invention may be used to screen for protease inhibitors which may act as antiviral agents. The structural and nonstructural regions of the HCV genome, including nucleotide and amino acid locations, have been determined, for example, as depicted in Houghton, M. (1996), Fig. 1; and Major, M.E. et al. (1997), Table 1.

Such above-mentioned protease inhibitors may take the form of chemical compounds or peptides which mimic the known cleavage sites of the protease and may be screened using methods known to those of skill in the art (Houghton, M. (1996) and Major, M.E. et al. (1997)). For example, a substrate may be employed which mimics the protease's natural substrate, but which provides a detectable signal (e.g. by fluorimetric or colorimetric methods) when cleaved. This substrate is then incubated with the protease and the candidate protease inhibitor under conditions of suitable pH, temperature etc. to detect protease activity. The proteolytic activities of the protease in the presence or absence of the candidate inhibitor are then determined.

In yet another embodiment, a candidate antiviral agent (such as a protease inhibitor) may be directly assayed in vivo for antiviral activity by administering the candidate antiviral agent to a chimpanzee transfected with a nucleic acid sequence of the invention and then measuring viral replication in vivo via methods such as RT-PCR. Of course, the chimpanzee may be treated with the candidate agent either before or after transfection with the infectious nucleic acid sequence so as to determine what stage, or stages, of viral infection and replication the agent is effective against.

The invention also provides that the nucleic acid sequences, viruses and polypeptides of the invention may be supplied in the form of a kit, alone or in the form of a pharmaceutical composition.

All scientific publication and/or patents cited herein are specifically incorporated by reference. The following examples illustrate various aspects of the invention but are in no way intended to limit the scope thereof.

EXAMPLES

MATERIALS AND METHODS

For Examples 1-4

Collection of Virus

Hepatitis C virus was collected and used as a source for the RNA used in generating the cDNA clones according to the present invention. Plasma containing strain H77 of HCV was obtained from a patient in the acute phase of transfusion-associated non-A, non-B hepatitis (Feinstone et al (1981)). Strain H77 belongs to genotype 1a of HCV (Ogata et al (1991), Inchauspe et al (1991)). The consensus sequence for most of its genome has been determined (Kolyakov et al (1996), Ogata et al (1991), Inchauspe et al (1991) and Farci et al (1996)).

RNA Purification

Viral RNA was collected and purified by conventional means. In general, total RNA from 10 μl of H77 plasma was extracted with the TRIzol system (GIBCO BRL). The RNA pellet was resuspended in 100 μl of 10 mM dithiothreitol (DTT) with 5% (vol/vol) RNasin (20 - 40 units/μl) (available from Promega) and 10 μl aliquots were stored at -80°C. In subsequent experiments RT-PCR was performed on RNA equivalent to 1 μl of H77 plasma, which contained an estimated 10^5 genome equivalents (GE) of HCV (Yanagi et al (1996)).

Primers used in the RT-PCR process were deduced from the genomic sequences of strain H77 according to procedures already known in the art (see above) or else were determined specifically for use herein. The primers generated for this purpose are listed in Table 1.

Table 1. **Oligonucleotides used for PCR amplification of strain H77 of HCV**

Designation	Sequence (5′ → 3′)*
H9261F	GGCTACAGCGGGGGAGACATTTATCACAGC
H3′X58R	TCATGCGGCTCACGGACCTTTCACAGCTAG
H9282F	**GTCCAAGCTT**ATCACAGCGTGTCTCATGCCCGGCCCCG
H3′X45R	**CGTCTCTAGA**GGACCTTTCACAGCTAGCCGTGACTAGGG
H9375F	TGAAGGTTGGGGTAAACACTCCGGCCTCTTAGGCCATT
H3′X-35R	ACATGATCTGCAGAGAGGCCAGTATCAGCACTCTC
H9386F	**GTCCAAGCTTACGCGT**AAACACTCCGGCCTCCTTAAGCCATTCCTG
H3′X-38R	**CGTCTCTAGA**CATGATCTGCAGAGAGGCCAGTATCAGCACTCTCTGC
H1	**TTTTTTTTTGCGGCCGC*TAATACGACTCACTATA***GCCAGCCCCCTGAT-GGGGGCGACACTCCACCATG
A1	ACTGTCTTCACGCAGAAAGCGTCTAGCCAT
H9417R	**CGTCTCTAGA**CAGGAAATGGCTTAAGAGGCCGGAGTGTTTACC

* HCV sequences are shown in plain text, non-HCV-specific sequences are shown in boldface and artificial cleavage sites used for cDNA cloning are underlined. The core sequence of the T7 promoter in primer H1 is shown in italics.

Primers for long RT-PCR were size-purified.

cDNA Synthesis

The RNA was denatured at 65°C for 2 min, and cDNA synthesis was performed in a 20 μl reaction volume with Superscript II reverse transcriptase (from GIBCO/BRL)

at 42 °C for 1 hour using specific antisense primers as described previously (Tellier et al (1996)). The cDNA mixture was treated with RNase H and RNase T1 (GIBCO/BRL) for 20 min at 37 °C.

Amplification and Cloning of the 3′ UTR

The 3′ UTR of strain H77 was amplified by PCR in two different assays. In both of these nested PCR reactions the first round of PCR was performed in a total volume of 50 μl in 1 x buffer, 250 μmol of each deoxynucleoside triphosphate (dNTP; Pharmacia), 20 pmol each of external sense and antisense primers, 1 μl of the Advantage KlenTaq polymerase mix (from Clontech) and 2 μl of the final cDNA reaction mixture. In the second round of PCR, 5 μl of the first round PCR mixture was added to 45 μl of PCR mixture prepared as already described. Each round of PCR (35 cycles), which was performed in a Perkin Elmer DNA thermal cycler 480, consisted of denaturation at 94 °C for 1 min (in 1st cycle 1 min 30 sec), annealing at 60°C for 1 min and elongation at 68°C for 2 min. In one experiment a region from NS5B to the conserved region of the 3′ UTR was amplified with the external primers H9261F and H3′X58R, and the internal primers H9282F and H3′X45R (Table 1). In another experiment, a segment of the variable region to the very end of the 3′ UTR was amplified with the external primers H9375F and H3′X-35R, and the internal primers H9386F and H3′X-38R (Table 1, Fig. 1). Amplified products were purified with QIAquick PCR purification kit (from QIAGEN), digested with *Hind* III and *Xba* I (from Promega), purified by either gel electrophoresis or phenol/chloroform extraction, and then cloned into the multiple cloning site of plasmid pGEM-9zf(-) (Promega) or pUC19 (Pharmacia). Cloning of cDNA into the vector was performed with T4 DNA ligase (Promega) by standard procedures.

Amplification of Near Full-Length H77 Genomes by Long PCR

The reactions were performed in a total volume of 50 μl in 1 x buffer, 250 μmol of each dNTP, 10 pmol each of sense and antisense primers, 1 μl of the Advantage KlenTaq polymerase mix and 2 μl of the cDNA reaction mixture (Tellier et al (1996)). A single PCR round of 35 cycles was performed in a Robocycler thermal cycler (from Stratagene), and consisted of denaturation at 99 °C for 35 sec, annealing at 67 °C for 30 sec and elongation at 68 °C for 10 min during the first 5 cycles, 11 min during the next 10 cycles, 12 min during the following 10 cycles and 13 min during the last 10 cycles. To amplify the complete ORF of HCV by long RT-PCR we used the sense primers H1 or A1 deduced from the 5' UTR and the antisense primer H9417R deduced from the variable region of the 3' UTR (Table 1, Fig. 1).

Construction of Full-Length H77 cDNA Clones

The long PCR products amplified with H1 and H9417R primers were cloned directly into pGEM-9zf(-) after digestion with *Not* I and *Xba* I (from Promega) (as per Fig. 1). Two clones were obtained with inserts of the expected size, pH21, and pH50,. Next, the chosen 3' UTR was cloned into both pH21, and pH50, after digestion with *Afl* II and *Xba* I (New England Biolabs). DH5α competent cells (GIBCO/BRL) were transformed and selected with LB agar plates containing 100 μg/ml ampicillin (from SIGMA). Then the selected colonies were cultured in LB liquid containing ampicillin at 30°C for ~18-20 hrs (transformants containing full-length or near full-length cDNA of H77 produced a very low yield of plasmid when cultured at 37 °C or for more than 24 hrs). After small scale preparation (Wizard Plus Minipreps DNA Purification Systems, Promega) each plasmid was retransformed to select a single clone, and large scale preparation of plasmid DNA was performed with a QIAGEN plasmid Maxi kit.

Cloning of Long RT-PCR Products Into a Cassette Vector

To improve the efficiency of cloning, a vector with consensus 5′ and 3′ termini of HCV strain H77 was constructed (Fig. 1). This cassette vector (pCV) was obtained by cutting out the *Bam*HI fragment (nts 1358 - 7530 of the H77 genome) from pH50, followed by religation. Next, the long PCR products of H77 amplified with H1 and H9417R or A1 and H9417R primers were purified (Geneclean spin kit; BIO 101) and cloned into pCV after digestion with *Age* I and *Afl* II (New England Biolabs) or with *Pin* AI (isoschizomer of *Age* I) and *Bfr* I (isoschizomer of Afl II) (Boehringer Mannheim). Large scale preparations of the plasmids containing full-length cDNA of H77 were performed as described above.

Construction of H77 Consensus Chimeric cDNA Clone

A full-length cDNA clone of H77 with an ORF encoding the consensus amino acid sequence was constructed by making a chimera from four of the cDNA clones obtained above. This consensus chimera, pCV-H77C, was constructed in two ligation steps by using standard molecular procedures and convenient cleavage sites and involved first a two piece ligation and then a three piece ligation. Large scale preparation of pCV-H77C was performed as already described.

In Vitro Transcription

Plasmids containing the full-length HCV cDNA were linearized with *Xba* I (from Promega), and purified by phenol/chloroform extraction and ethanol precipitation. A 100 μl reaction mixture containing 10 μg of linearized plasmid DNA, 1 x transcription buffer, 1 mM ATP, CTP, GTP and UTP, 10mM DTT , 4% (v/v) RNasin (20-40 units/μl) and 2 μl of T7 RNA polymerase (Promega) was incubated at 37 °C for 2 hrs. Five μl of the reaction mixture was analyzed by agarose gel electrophoresis followed by ethidium bromide staining. The transcription reaction mixture was diluted with 400 μl of ice-cold phosphate-buffered saline

without calcium or magnesium, immediately frozen on dry ice and stored at -80 °C. The final nucleic acid mixture was injected into chimpanzees within 24 hrs.

Intrahepatic Transfection of Chimpanzees

Laparotomy was performed and aliquots from two transcription reactions were injected into 6 sites of the exposed liver (Emerson et al (1992). Serum samples were collected weekly from chimpanzees and monitored for liver enzyme levels and anti-HCV antibodies. Weekly samples of 100 μl of serum were tested for HCV RNA in a highly sensitive nested RT-PCR assay with AmpliTaq Gold (Perkin Elmer)(Yanagi et al (1996); Bukh et al (1992)). The genome titer of HCV was estimated by testing 10-fold serial dilutions of the extracted RNA in the RT-PCR assay (Yanagi et al (1996)). The two chimpanzees used in this study were maintained under conditions that met all requirements for their use in an approved facility.

The consensus sequence of the complete ORF from HCV genomes recovered at week 2 post inoculation (p.i) was determined by direct sequencing of PCR products obtained in long RT-PCR with primers A1 and H9417R followed by nested PCR of 10 overlapping fragments. The consensus sequence of the variable region of the 3' UTR was determined by direct sequencing of an amplicon obtained in nested RT-PCR as described above. Finally, we amplified selected regions independently by nested RT-PCR with AmpliTaq Gold.

Sequence Analysis

Both strands of DNA from PCR products, as well as plasmids, were sequenced with the ABI PRISM Dye Termination Cycle Sequencing Ready Reaction Kit using Taq DNA polymerase (Perkin Elmer) and about 100 specific sense and antisense sequence primers.

The consensus sequence of HCV strain H77 was determined in two different ways. In one approach, overlapping PCR products were directly sequenced, and

amplified in nested RT-PCR from the H77 plasma sample. The sequence analyzed (nucleotides (nts) 35-9417) included the entire genome except the very 5' and 3' termini. In the second approach, the consensus sequence of nts 157-9384 was deduced from the sequences of 18 full-length cDNA clones.

EXAMPLE 1

Variability in the sequence of the 3' UTR of HCV strain H77

The heterogeneity of the 3' UTR was analyzed by cloning and sequencing of DNA amplicons obtained in nested RT-PCR. 19 clones containing sequences of the entire variable region, the poly U-UC region and the adjacent 19 nt of the conserved region, and 65 clones containing sequences of the entire poly U-UC region and the first 63 nts of the conserved region were analyzed. This analysis confirmed that the variable region consisted of 43 nts, including two conserved termination codons (Han et al (1992)). The sequence of the variable region was highly conserved within H77 since only 3 point mutations were found among the 19 clones analyzed. A poly U-UC region was present in all 84 clones analyzed. However, its length varied from 71-141 nts. The length of the poly U region was 9-103 nts, and that of the poly UC region was 35-85 nts. The number of C residues increased towards the 3' end of the poly UC region but the sequence of this region is not conserved. The first 63 nts of the conserved region were highly conserved among the clones analyzed, with a total of only 14 point mutations. To confirm the validity of the analysis, the 3' UTR was reamplified directly from a full-length cDNA clone of HCV (see below) by the nested-PCR procedure with the primers in the variable region and at the very 3' end of the HCV genome and cloned the PCR product. Eight clones had 1-7 nt deletions in the poly U region. Furthermore, although the C residues of the poly UC region were maintained, the

spacing of these varied because of 1-2 nt deletions of U residues. These deletions must be artifacts introduced by PCR and such mistakes may have contributed to the heterogeneity originally observed in this region. However, the conserved region of the 3′ UTR was amplified correctly, suggesting that the deletions were due to difficulties in transcribing a highly repetitive sequence.

One of the 3′ UTR clones was selected for engineering of full-length cDNA clones of H77. This clone had the consensus variable sequence except for a single point mutation introduced to create an Afl II cleavage site, a poly U-UC stretch of 81 nts with the most commonly observed UC pattern and the consensus sequence of the complete conserved region of 101 nts, including the distal 38 nts which originated from the antisense primer used in the amplification. After linearization with Xba I, the DNA template of this clone had the authentic 3′ end.

EXAMPLE 2

The Entire Open Reading Frame of H77 Amplified in One Round of Long RT-PCR

It had been previously demonstrated that a 9.25 kb fragment of the HCV genome from the 5' UTR to the 3′ end of NS5B could be amplified from 10^4 GE (genome equivalents) of H77 by a single round of long RT-PCR (Tellier et al (1996a)). In the current study, by optimizing primers and cycling conditions, the entire ORF of H77 was amplified in a single round of long RT-PCR with primers from the 5′ UTR and the variable region of the 3′ UTR. In fact, 9.4 kb of the H77 genome (H product: from the very 5′ end to the variable region of the 3′ UTR) could be amplified from 10^5GE or 9.3 kb (A product: from within the 5′ UTR to the variable region of the 3′ UTR) from 10^4GE or 10^5GE, in a single round of long RT-PCR (Fig. 2). The PCR products amplified from 10^5GE of H77 were used for engineering full-length cDNA clones (see below).

EXAMPLE 3

Construction of Multiple Full-Length cDNA Clones of H77 in a Single Step by Cloning of Long RT-PCR Amplicons Directly into a Cassette Vector with Fixed 5' and 3' Termini

Direct cloning of the long PCR products (H), which contained a 5' T7 promoter, the authentic 5' end, the entire ORF of H77 and a short region of the 3' UTR, into pGEM-9zf(-) vector by *Not* I and *Xba* I digestion was first attempted. However, among the 70 clones examined all but two had inserts that were shorter than predicted. Sequence analysis identified a second *Not* I site in the majority of clones, which resulted in deletion of the nts past position 9221. Only two clones (pH21$_{I}$ and pH50$_{I}$) were missing the second Not I site and had the expected 5' and 3' sequences of the PCR product. Therefore, full-length cDNA clones (pH21 and pH50) were constructed by inserting the chosen 3' UTR into pH21$_{I}$ and pH50$_{I}$, respectively. Sequence analysis revealed that clone pH21 had a complete full-length sequence of H77; this clone was tested for infectivity. The second clone, pH50, had one nt deletion in the ORF at position 6365; this clone was used to make a cassette vector.

The complete ORF was amplified by constructing a cassette vector with fixed 5' and 3' termini as an intermediate of the full-length cDNA clones. This vector (pCV) was constructed by digestion of clone pH50 with *Bam*HI, followed by religation, to give a shortened plasmid readily distinguished from plasmids containing the full-length insert. Attempts to clone long RT-PCR products (H) into pCV by *Age* I and *Afl* II yielded only 1 of 23 clones with an insert of the expected size. In order to increase the efficiency of cloning, we repeated the procedure but used *Pin* A I and *Bfr* I instead of the respective isoschizomers *Age* I and *Afl* II. By this protocol, 24 of 31 H clones and 30 of 35 A clones had the full-length cDNA

of H77 as evaluated by restriction enzyme digestion. A total of 16 clones, selected at random, were each retransformed, and individual plasmids were purified and completely sequenced.

EXAMPLE 4

Demonstration of Infectious Nature of Transcripts of a cDNA Clone Representing the Consensus Sequence of Strain H77

A consensus chimera was constructed from 4 of the full-length cDNA clones with just 2 ligation steps. The final construct, pCV-H77C, had 11 nt differences from the consensus sequence of H77 in the ORF (Fig. 3). However, 10 of these nucleotide differences represented silent mutations. The chimeric clone differed from the consensus sequence at only one amino acid [L instead of F at position 790]. Among the 18 ORFs analyzed above, the F residue was found in 11 clones and the L residue in 7 clones. However, the L residue was dominant in other isolates of genotype 1a, including a first passage of H77 in a chimpanzee (Inchauspe et al (1991)).

To test the infectivity of the consensus chimeric clone of H77 intrahepatic transfection of a chimpanzee was performed. The pCV-H77C clone was linearized with Xba I and transcribed *in vitro* by T7 RNA polymerase (Fig. 2). The transcription mixture was next injected into 6 sites of the liver of chimpanzee 1530. The chimpanzee became infected with HCV as measured by detection of 10^2 GE/ml of viral genome at week 1 p.i. Furthermore, the HCV titer increased to 10^4GE/ml at week 2 p.i., and reached 10^6 GE/ml by week 8 p.i. The viremic pattern observed in the early phase of the infection with the recombinant virus was similar to that observed in chimpanzees inoculated intravenously with strain H77 or other strains of HCV (Shimizu (1990)).

The sequence of the HCV genomes from the serum sample collected at week 2 p.i. was analyzed. The

consensus sequence of nts 298-9375 of the recovered genomes was determined by direct sequencing of PCR products obtained in long RT-PCR followed by nested PCR of 10 overlapping fragments. The identity to clone pCV-H77C sequence was 100%. The consensus sequence of nts 96-291,1328-1848, 3585-4106, 4763-5113 and 9322-9445 was determined from PCR products obtained in different nested RT-PCR assays. The identity of these sequences with pCV-H77C was also 100%. These latter regions contained 4 mutations unique to the consensus chimera, including the artificial Afl II cleavage site in the 3' UTR. Therefore, RNA transcripts of this clone of HCV were infectious.

The infectious nature of the consensus chimera indicates that the regions of the 5' and 3' UTRs incorporated into the cassette vector do not destroy viability. This makes it highly advantageous to use the cassette vector to construct infectious cDNA clones of other HCV strains when the consensus sequence for each ORF is inserted.

In addition, two complete full-length clones (dubbed pH21 and pCV-H11) constructed were not infectious, as shown by intrahepatic injection of chimpanzees with the corresponding RNA transcripts. Thus, injection of the transcription mixture into 3 sites of the exposed liver resulted in no observable HCV replication and weekly serum samples were negative for HCV RNA at weeks 1 - 17 p.i. in a highly sensitive nested RT-PCR assay. The cDNA template injected along with the RNA transcripts was also not detected in this assay.

Moreover, the chimpanzee remained negative for antibodies to HCV throughout the follow-up. Subsequent sequence analysis revealed that 7 of 16 additional clones were defective for polyprotein synthesis and all clones had multiple amino acid mutations compared with the consensus sequence of the parent strain. For example, clone pH21, which was not infectious, had 7 amino acid

substitutions in the entire predicted polyprotein compared with the consensus sequence of H77 (Fig. 3). The most notable mutation was at position 1026, which changed L to Q, altering the cleavage site between NS2 and NS3 (Reed (1995)). Clone pCV-H11, also non-infectious, had 21 amino acid substitutions in the predicted polyprotein compared with the consensus sequence of H77 (Fig. 3). The amino acid mutation at position 564 eliminated a highly conserved C residue in the E2 protein (Okamoto (1992a)).

EXAMPLE 4A

The chimpanzee of Example 4, designated 1530, was monitored out to 32 weeks p.i. for serum enzyme levels (ALT) and the presence of anti-HCV antibodies, HCV RNA, and liver histopathology. The results are shown in Figure **18B**.

A second chimp, designated 1494, was also transfected with RNA transcripts of the pCV-H77C clone and monitored out to 17 weeks p.i. for the presence of anti-HCV antibodies, HCV RNA and elevated serum enzyme levels. The results are shown in Figure **18A**.

MATERIALS AND METHODS
for Examples 5-10

Source Of HCV Genotype 1b

An infectious plasma pool (second chimpanzee passage) containing strain HC-J4, genotype 1b, was prepared from acute phase plasma of a chimpanzee experimentally infected with serum containing HC-J4/91 (Okamoto et al., 1992b). The HC-J4/91 sample was obtained from a first chimpanzee passage during the chronic phase of hepatitis C about 8 years after experimental infection. The consensus sequence of the entire genome, except for the very 3′ end, was determined previously for HC-J4/91 (Okamoto et al., 1992b).

Preparation Of HCV RNA

Viral RNA was extracted from 100 μl aliquots of the HC-J4 plasma pool with the TRIzol system (GIBCO BRL).

The RNA pellets were each resuspended in 10 μl of 10 mM dithiothreitol (DTT) with 5% (vol/vol) RNasin (20-40 units/μl) (Promega) and stored at -80°C or immediately used for cDNA synthesis.

Amplification And Cloning Of The 3′ UTR

A region spanning from NS5B to the conserved region of the 3′ UTR was amplified in nested RT-PCR using the procedure of Yanagi et al., (1997).

In brief, the RNA was denatured at 65°C for 2 minutes, and cDNA was synthesized at 42°C for 1 hour with Superscript II reverse transcriptase (GIBCO BRL) and primer H3′X58R (Table 1) in a 20 μl reaction volume. The cDNA mixture was treated with RNase H and RNase T1 (GIBCO BRL) at 37°C for 20 minutes. The first round of PCR was performed on 2 μl of the final cDNA mixture in a total volume of 50 μl with the Advantage cDNA polymerase mix (Clontech) and external primers H9261F (Table 1) and H3′X58R (Table 1). In the second round of PCR [internal primers H9282F (Table 1) and H3′X45R (Table 1)], 5 μl of the first round PCR mixture was added to 45 μl of the PCR reaction mixture. Each round of PCR (35 cycles), was performed in a DNA thermal cycler 480 (Perkin Elmer) and consisted of denaturation at 94°C for 1 minute (1st cycle: 1 minute 30 sec), annealing at 60°C for 1 minute and elongation at 68°C for 2 minutes. After purification with QIAquick PCR purification kit (QIAGEN), digestion with *Hin*dIII and *Xba*I (Promega), and phenol/chloroform extraction, the amplified products were cloned into pGEM-9zf(-) (Promega) (Yanagi et al., 1997).

Amplification And Cloning Of The Entire ORF

A region from within the 5′ UTR to the variable region of the 3′ UTR of strain HC-J4 was amplified by long RT-PCR (Fig. 1) (Yanagi et al., 1997). The cDNA was synthesized at 42°C for 1 hour in a 20 μl reaction volume with Superscript II reverse transcriptase and primer J4-

9405R (5'-GCCTATTGGCCTGGAGTGGTTAGCTC-3'), and treated with RNases as above. The cDNA mixture (2 μl) was amplified by long PCR with the Advantage cDNA polymerase mix and primers A1 (Table 1) (Bukh et al., 1992; Yanagi et al., 1997) and J4-9398R (5'-***AGGATGGC***<u>CTTAAG</u>GCCTGGAGTGGTTAGCTCCCCGTTCA-3'). Primer J4-9398R contained extra bases (***bold***) and an artificial *Afl*II cleavage site (underlined). A single PCR round was performed in a Robocycler thermal cycler (Stratagene), and consisted of denaturation at 99°C for 35 seconds, annealing at 67°C for 30 seconds and elongation at 68°C for 10 minutes during the first 5 cycles, 11 minutes during the next 10 cycles, 12 minutes during the following 10 cycles and 13 minutes during the last 10 cycles.

After digesting the long PCR products obtained from strain HC-J4 with *Pin*AI (isoschizomer of *Age*I) and *Bfr*I (isoschizomer of *Afl*II) (Boehringer Mannheim), attempts were made to clone them directly into a cassette vector (pCV), which contained the 5' and 3' termini of strain H77 (Figure 1) but no full-length clones were obtained. Accordingly, to improve the efficiency of cloning, the PCR product was further digested with *Bgl*II (Boehringer Mannheim) and the two resultant genome fragments [L fragment: *Pin*AI/*Bgl*II, nts 156 - 8935; S fragment: *Bgl*II/*Brf*I, nts 8936 - 9398] were separately cloned into pCV (Figure 6).

DH5α competent cells (GIBCO BRL) were transformed and selected on LB agar plates containing 100 μg/ml ampicillin (SIGMA) and amplified in LB liquid cultures at 30°C for 18-20 hours.

Sequence analysis of 9 plasmids containing the S fragment (miniprep samples) and 9 plasmids containing the L fragment (maxiprep samples) were performed as described previously (Yanagi et al., 1997). Three L fragments, each encoding a distinct polypeptide, were cloned into pCV-J4S9 (which contained an S fragment encoding the consensus

amino acid sequence of HC-J4) to construct three chimeric full-length HCV cDNAs (pCV-J4L2S, pCV-J4L4S and pCV-J4L6S) (Fig. 6). Large scale preparation of each clone was performed as described previously with a QIAGEN plasmid Maxi kit (Yanagi et al., 1997) and the authenticity of each clone was confirmed by sequence analysis.

Sequence Analysis

Both strands of DNA were sequenced with the ABI PRISM Dye Termination Cycle Sequencing Ready Reaction Kit using Taq DNA polymerase (Perkin Elmer) and about 90 specific sense and antisense primers. Analyses of genomic sequences, including multiple sequence alignments and tree analyses, were performed with GeneWorks (Oxford Molecular Group) (Bukh et al., 1995).

The consensus sequence of strain HC-J4 was determined by direct sequencing of PCR products (nts 11 - 9412) and by sequence analysis of multiple cloned L and S fragments (nts 156 -9371). The consensus sequence of the 3′ UTR (3′ variable region, polypyrimidine tract and the first 16 nucleotides of the conserved region) was determined by analysis of 24 cDNA clones.

Intrahepatic Transfection Of A Chimpanzee With Transcribed RNA

Two in vitro transcription reactions were performed with each of the three full-length clones. In each reaction 10 μg of plasmid DNA linearized with *Xba* I (Promega) was transcribed in a 100 μl reaction volume with T7 RNA polymerase (Promega) at 37°C for 2 hours as described previously (Yanagi et al., 1997). Five μl of the final reaction mixture was analyzed by agarose gel electrophoresis and ethidium bromide staining (Fig. 5). Each transcription mixture was diluted with 400 μl of ice-cold phosphate-buffered saline without calcium or magnesium and then the two aliquots from the same cDNA clone were combined, immediately frozen on dry ice and stored at -80°C. Within 24 hours after freezing the

transcription mixtures were injected into the chimpanzee by percutaneous intrahepatic injection that was guided by ultrasound. Each inoculum was individually injected (5-6 sites) into a separate area of the liver to prevent complementation or recombination. The chimpanzee was maintained under conditions that met all requirements for its use in an approved facility.

Serum samples were collected weekly from the chimpanzee and monitored for liver enzyme levels and anti-HCV antibodies. Weekly samples of 100 μl of serum were tested for HCV RNA in a sensitive nested RT-PCR assay (Bukh et al., 1992, Yanagi et al., 1996) with AmpliTaq Gold DNA polymerase. The genome equivalent (GE) titer of HCV was determined by testing 10-fold serial dilutions of the extracted RNA in the RT-PCR assay (Yanagi et al., 1996) with 1 GE defined as the number of HCV genomes present in the highest dilution which was positive in the RT-nested PCR assay.

To identify which of the three clones was infectious *in vivo*, the NS3 region (nts 3659 - 4110) from the chimpanzee serum was amplified in a highly sensitive and specific nested RT-PCR assay with AmpliTaq Gold DNA polymerase and the PCR products were cloned with a TA cloning kit (Invitrogen). In addition, the consensus sequence of the nearly complete genome (nts 11 - 9441) was determined by direct sequencing of overlapping PCR products.

EXAMPLE 5

Sequence Analysis Of Infectious Plasma Pool Of Strain HC-J4 Used As The Cloning Source

As an infectious cDNA clone of a genotype 1a strain of HCV had been obtained only after the ORF was engineered to encode the consensus polypeptide (Kolykhalov et al., 1997; Yanagi et al., 1997), a detailed sequence analysis of the cloning source was performed to determine

the consensus sequence prior to constructing an infectious cDNA clone of a 1b genotype.

A plasma pool of strain HC-J4 was prepared from acute phase plasmapheresis units collected from a chimpanzee experimentally infected with HC-J4/91 (Okamoto et al., 1992b). This HCV pool had a PCR titer of 10^4 - 10^5 GE/ml and an infectivity titer of approximately 10^3 chimpanzee infectious doses per ml.

The heterogeneity of the 3' UTR of strain HC-J4 was determined by analyzing 24 clones of nested RT-PCR product. The consensus sequence was identical to that previously published for HC-J4/91 (Okamoto et al., 1992b), except at position 9407 (see below). The variable region consisted of 41 nucleotides (nts. 9372 - 9412), including two in-frame termination codons. Furthermore, its sequence was highly conserved except at positions 9399 (19 A and 5 T clones) and 9407 (17 T and 7 A clones). The poly U-UC region varied slightly in composition and greatly in length (31-162 nucleotides). In the conserved region, the first 16 nucleotides of 22 clones were identical to those previously published for other genotype 1 strains, whereas two clones each had a single point mutation. These data suggested that the structural organization at the 3' end of HC-J4 was similar to that of the infectious clone of a genotype 1a strain of Yanagi et al (1997).

Next, the entire ORF of HC-J4 was amplified in a single round of long RT-PCR (Figure **5**). The original plan was to clone the resulting PCR products into the *Pin*AI and *Brf*I site of a HCV cassette vector (pCV), which had fixed 5' and 3' termini of genotype 1a (Yanagi et al., 1997) but since full-length clones were not obtained, two genome fragments (L and S) derived from the long RT-PCR products (Figure **6**) were separately subcloned into pCV.

To determine the consensus sequence of the ORF, the sequence of 9 clones each of the L fragment (pCV-J4L)

and of the S fragment (pCV-J4S) was determined and quasispecies were found at 275 nucleotide (3.05 %) and 78 amino acid (2.59 %) positions, scattered throughout the 9030 nts (3010 aa) of the ORF (Figure 7). Of the 161 nucleotide substitutions unique to a single clone, 71% were at the third position of the codon and 72 % were silent.

Each of the nine L clones represented the near complete ORF of an individual genome. The differences among the L clones were 0.30 - 1.53% at the nucleotide and 0.31 - 1.47% at the amino acid level (Figure 8). Two clones, L1 and L7, had a defective ORF due to a single nucleotide deletion and a single nucleotide insertion, respectively. Even though the HC-J4 plasma pool was obtained in the early acute phase, it appeared to contain at least three viral species (Figure 9). Species A contained the L1, L2, L6, L8 and L9 clones, species B the L3, L7 and L10 clones and species C the L4 clone. Although each species A clone was unique all A clones differed from all B clones at the same 20 amino acid sites and at these positions, species C had the species A sequence at 14 positions and the species B sequence at 6 positions (Figure 7).

Okamoto and coworkers (Okamoto et al., 1992b) previously determined the nearly complete genome consensus sequence of strain HC-J4 in acute phase serum of the first chimpanzee passage (HC-J4/83) as well as in chronic phase serum collected 8.2 years later (HC-J4/91). In addition, they determined the sequence of amino acids 379 to 413 (including HVR1) and amino acids 468 to 486 (including HVR2) of multiple individual clones (Okamoto et al., 1992b).

It was found by the present inventors that the sequences of individual genomes in the plasma pool collected from a chimpanzee inoculated with HC-J4/91 were all more closely related to HC-J4/91 than to HC-J4/83

(Figures 8, 9) and contained HVR amino acid sequences closely related to three of the four viral species previously found in HC-J4/91 (Figure 10).

Thus, the data presented herein demonstrate the occurrence of the simultaneous transmission of multiple species to a single chimpanzee and clearly illustrates the difficulties in accurately determining the evolution of HCV over time since multiple species with significant changes throughout the HCV genome can be present from the onset of the infection. Accordingly, infection of chimpanzees with monoclonal viruses derived from the infectious clones described herein will make it possible to perform more detailed studies of the evolution of HCV *in vivo* and its importance for viral persistence and pathogenesis.

EXAMPLE 6

Determination Of The Consensus Sequence Of HC-J4 In The Plasma Pool

The consensus sequence of nucleotides 156-9371 of HC-J4 was determined by two approaches. In one approach, the consensus sequence was deduced from 9 clones of the long RT-PCR product. In the other approach the long RT-PCR product was reamplified by PCR as overlapping fragments which were sequenced directly. The two "consensus" sequences differed at 31 (0.34%) of 9216 nucleotide positions and at 11 (0.37%) of 3010 deduced amino acid positions (Figure 7). At all of these positions a major quasispecies of strain HC-J4 was found in the plasma pool. At 9 additional amino acid positions the cloned sequences displayed heterogeneity and the direct sequence was ambiguous (Figure 7). Finally, it should be noted that there were multiple amino acid positions at which the consensus sequence obtained by direct sequencing was identical to that obtained by cloning and sequencing even though a major quasispecies

was detected (Figure 7).

For positions at which the two "consensus" sequences of HC-J4 differed, both amino acids were included in a composite consensus sequence (Figure 7). However, even with this allowance, none of the 9 L clones analyzed (aa 1 - 2864) had the composite consensus sequence: two clones did not encode the complete polypeptide and the remaining 7 clones differed from the consensus sequence by 3 - 13 amino acids (Figure 7).

EXAMPLE 7

Construction Of Chimeric Full-Length cDNA Clones Containing The Entire ORF Of HC-J4

The cassette vector used to clone strain H77 was used to construct an infectious cDNA clone containing the ORF of a second subtype.

In brief, three full-length cDNA clones were constructed by cloning different L fragments into the *Pin*AI/*Bgl*II site of pCV-J4S9, the cassette vector for genotype 1a (Figure 6), which also contained an S fragment encoding the consensus amino acid sequence of HC-J4. Therefore, although the ORF was from strain HC-J4, most of the 5′ and 3′ terminal sequences originated from strain H77. As a result, the 5′ and 3′ UTR were chimeras of genotypes 1a and 1b (Figure 11).

The first 155 nucleotides of the 5′ UTR were from strain H77 (genotype 1a), and differed from the authentic sequence of HC-J4 (genotype 1b) at nucleotides 11, 12, 13, 34 and 35. In two clones (pCV-J4L2S, pCV-J4L6S) the rest of the 5′ UTR had the consensus sequence of HC-J4, whereas the third clone (pCV-J4L4S) had a single nucleotide insertion at position 207. In all 3 clones the first 27 nucleotides of the 3′ variable region of the 3′ UTR were identical with the consensus sequence of HC-J4. The remaining 15 nucleotides of the variable region, the poly U-UC region and the 3′ conserved region of the 3′ UTR

had the same sequence as an infectious clone of strain H77 (Figure **11**).

None of the three full-length clones of HC-J4 had the ORF composite consensus sequence (Figures **7, 12**). The pCV-J4L6S clone had only three amino acid changes: Q for R at position 231 (E1), V for A at position 937 (NS2) and T for S at position 1215 (NS3). The pCV-J4L4S clone had 7 amino acid changes, including a change at position 450 (E2) that eliminated a highly conserved N-linked glycosylation site (Okamoto et al., 1992a). Finally, the pCV-J4L2S clone had 9 amino acid changes compared with the consensus sequence of HC-J4. A change at position 304 (E1) mutated a highly conserved cysteine residue (Bukh et al., 1993; Okamoto et al., 1992a).

EXAMPLE 8

Transfection Of A Chimpanzee By In Vitro Transcripts Of A Chimeric cDNA

The infectivity of the three chimeric HCV clones was determined by ultra-sound-guided percutaneous intrahepatic injection into the liver of a chimpanzee of the same amount of cDNA and transcription mixture for each of the clones (Figure **5**). This procedure is a less invasive procedure than the laparotomy procedure utilized by Kolykhalov et al. (1997) and Yanagi et al. (1997) and should facilitate in vivo studies of cDNA clones of HCV in chimpanzees since percutaneous procedures, unlike laparotomy, can be performed repeatedly.

As shown in Figure **13**, the chimpanzee became infected with HCV as measured by increasing titers of 10^2 GE/ml at week 1 p.i., 10^3 GE/ml at week 2 p.i. and 10^4 - 10^5 GE/ml during weeks 3 to 10 p.i.

The viremic pattern found in the early phase of the infection was similar to that observed for the recombinant H77 virus in chimpanzees (Bukh et al., unpublished data; Kolykhalov et al., 1997; Yanagi et al.,

1997). The chimpanzee transfected in the present study was chronically infected with hepatitis G virus (HGV/GBV-C) (Bukh et al., 1998) and had a titer of 10^6 GE/ml at the time of HCV transfection. Although HGV/GBV-C was originally believed to be a hepatitis virus, it does not cause hepatitis in chimpanzees (Bukh et al., 1998) and may not replicate in the liver (Laskus et al., 1997). The present study demonstrated that an ongoing infection of HGV/GBV-C did not prevent acute HCV infection in the chimpanzee model.

However, to identify which of the three full-length HC-J4 clones were infectious, the NS3 region (nts. 3659 - 4110) of HCV genomes amplified by RT-PCR from serum samples taken from the infected chimpanzee during weeks 2 and 4 post-infection (p.i.) were cloned and sequenced. As the PCR primers were a complete match with each of the original three clones, this assay should not have preferentially amplified one virus over another. Sequence analysis of 26 and 24 clones obtained at weeks 2 and 4 p.i., respectively, demonstrated that all originated from the transcripts of pCV-J4L6S.

Moreover, the consensus sequence of PCR products of the nearly complete genome (nts. 11-9441), amplified from serum obtained during week 2 p.i., was identical to the sequence of pCV-J4L6S and there was no evidence of quasispecies. Thus, RNA transcripts of pCV-J4L6S, but not of pCV-J4L2S or pCV-J4L4S, were infectious in vivo. The data in Figure **13** is therefore the product of the transfection of RNA transcripts of pCV-J4L6S.

In addition, the chimeric sequences of genotypes 1a and 1b in the UTRs were maintained in the infected chimpanzee. The consensus sequence of nucleotides 11 - 341 of the 5' UTR and the variable region of the 3' UTR, amplified from serum obtained during weeks 2 and 4 p.i., had the expected chimeric sequence of genotypes 1a and 1b (Fig. 11). Also three of four clones of the 3' UTR

obtained at week 2 p.i. had the chimeric sequence of the variable region, whereas a single substitution was noted in the fourth clone. However, in all four clones the poly U region was longer (2-12 nts) than expected. Also, extra C and G residues were observed in this region. For the most part, the number of C residues in the poly UC region was maintained in all clones although the spacing varied. As shown previously, variations in the number of U residues can reflect artifacts introduced during PCR amplification (Yanagi et al., 1997). The sequence of the first 19 nucleotides of the conserved region was maintained in all four clones. Thus, with the exception of the poly U-UC region, the genomic sequences recovered from the infected chimpanzee were exactly those of the chimeric infectious clone pCV-J4BL6S.

The results presented in Figure **13** therefore demonstrate that HCV polypeptide sequences other than the consensus sequence can be infectious and that a chimeric genome containing portions of the H77 termini could produce an infectious virus. In addition, these results showed for the first time that it is possible to make infectious viruses containing 5′ and 3′ terminal sequences specific for two different subtypes of the same major genotype of HCV.

EXAMPLE 9

Construction Of A Chimeric 1a/1b Infectious Clone

A chimeric 1a/1b infectious clone in which the structural region of the genotype 1b infectious clone is inserted into the 1a clone of Yanagi et al. (1997) is constructed by following the protocol shown in Figure **15**. The resultant chimera contains nucleotides 156-2763 of the 1b clone described herein inserted into the 1a clone of Figures **4A-4F**. The sequences of the primers shown in Figure **15** which are used in constructing this chimeric clone, designated pH77CV-J4, are presented below.

1. <u>H2751S (Cla I/*Nde* I)</u>

CGT CAT CGA TCC TCA GCG GGC ATA TGC ACT GGA CAC GGA

2. <u>H2870R</u>

CAT GCA CCA GCT GAT ATA GCG CTT GTA ATA TG

3. <u>H7851S</u>

TCC GTA GAG GAA GCT TGC AGC CTG ACG CCC

4. <u>H9173 R(P-M)</u>

GTA CTT GCC ACA TAT AGC AGC CCT GCC TCC TCT G

5. <u>H9140S (P-M)</u>

CAG AGG AGG CAG GGC TGC TAT ATG TGG CAA GTA C

6. <u>H9417R</u>

CGT CTC TAG ACA GGA AAT GGC TTA AGA GGC CGG AGT GTT TAC C

7. <u>J4-2271S</u>

TGC AAT TGG ACT CGA GGA GAG CGC TGT AAC TTG GAG

8. <u>J4-2776R (Nde I)</u>

CGG TCC AAG GCA TAT GCT CGT GGT GGT AAC GCC AG

Transcripts of the chimeric 1a/1b clone (whose sequence is shown in Figures **16A-16F**) are then produced and transfected into chimpanzees by the methods described in the Materials and Methods section herein and the transfected animals are then be subjected to biochemical (ALT levels), histopathological and PCR analyses to determine the infectivity of the chimeric clone.

<u>EXAMPLE 10</u>

Construction of 3' Deletion Mutants <u>Of The 1a Infectious Clone pCV-H77C</u>

Seven constructs having various deletions in the 3' untranslated region (UTR) of the 1a infectious clone pCV-H77C were constructed as described in Figures **17A-17B**. The 3' untranslated sequence remaining in each of the seven constructs following their respective deletions is shown in Figures **17A-17B**.

Construct pCV-H77C(-98X) containing a deletion of the 3'-most 98 nucleotide sequences in the 3'-UTR was transcribed <u>in vitro</u> according to the methods described

herein and 1 ml of the diluted transcription mixture was percutaneously transfected into the liver of a chimpanzee with the aid of ultrasound. After three weeks, the transfection was repeated. The chimpanzee was observed to be negative for hepatitis C virus replication as measured by RT-PCR assay for 5 weeks after transfection. These results demonstrate that the deleted 98 nucleotide 3'-UTR sequence was critical for production of infectious HCV and appear to contradict the reports of Dash et al. (1996) and Yoo et al. (1995) who reported that RNA transcripts from cDNA clones of HCV-1 and HCV-N lacking the terminal 98 conserved nucleotides at the very 3' end of the 3'-UTR resulted in viral replication after transfection into human hematoma cell lines.

Transcripts of the (-42X) mutant (Figure **17C**) were also produced and transfected into a chimpanzee and transcripts of the other five deletion mutants shown in Figures **17D-17G**) are to be produced and transfected into chimpanzees by the methods described herein. All transfected animals are to then be assayed for viral replication via RT-PCR.

Discussion

In two recent reports on transfection of chimpanzees, only those clones engineered to have the independently determined and slightly different consensus amino acid sequence of the polypeptide of strain H77 were infectious (Kolykhalov et al., 1997; Yanagi et al., 1997). Although the two infectious clones differed at four amino acid positions, these differences were represented in a major component of the quasispecies of the cloning source. In the present study, a single consensus sequence of strain HC-J4 could not be defined because the consensus sequence obtained by two different approaches (direct sequencing and sequencing of cloned products) differed at 20 amino acid positions, even though the same genomic PCR product was analyzed. The infectious clone differed at

two positions from the composite amino acid consensus sequence, from the sequence of the 8 additional HC-J4 clones analyzed in this study and from published sequences of earlier passage samples. An additional amino acid differed from the composite consensus sequence but was found in two other HC-J4 clones analyzed in this study. The two non-infectious full-length clones of HC-J4 differed from the composite consensus sequence by only 7 and 9 amino acid differences. However, since these clones had the same termini as the infectious clone (except for a single nucleotide insertion in the 5' UTR of pCV-J4L4S), one or more of these amino acid changes in each clone was apparently deleterious for the virus.

It was also found in the present study that HC-J4, like other strains of genotype 1b (Kolykhalov et al., 1996; Tanaka et al., 1996; Yamada et al., 1996), had a poly U-UC region followed by a terminal conserved element. The poly U-UC region appears to vary considerably so it was not clear whether changes in this region would have a significant effect on virus replication. On the other hand, the 3' 98 nucleotides of the HCV genome were previously shown to be identical among other strains of genotypes 1a and 1b (Kolykhalov et al., 1996; Tanaka et al., 1996). Thus, use of the cassette vector would not alter this region except for addition of 3 nucleotides found in strain H77 between the poly UC region and the 3' 98 conserved nucleotides.

In conclusion, an infectious clone representing a genotype 1b strain of HCV has been constructed. Thus, it has been demonstrated that it was possible to obtain an infectious clone of a second strain of HCV. In addition, it has been shown that a consensus amino acid sequence was not absolutely required for infectivity and that chimeras between the UTRs of two different genotypes could be viable.

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WHAT IS CLAIMED IS:

1. A purified and isolated nucleic acid molecule which encodes human hepatitis C virus, said molecule capable of expressing said virus when transfected into cells.

2. The nucleic acid molecule of claim 1, wherein said molecule encodes the amino acid sequence shown in Figures 14G-14H.

3. The nucleic acid molecule of claim 2, wherein said molecule comprises the nucleic acid sequence shown in Figures 14A-14F.

4. The nucleic acid molecule acid molecule of claim 1, wherein said molecule encodes the amino acid sequence shown in Figures 4G-4H.

5. The nucleic acid molecule of claim 4, wherein said molecule comprises the nucleic acid sequence shown in Figures 4A-4F.

6. The nucleic acid molecule of claim 1, wherein a fragment of said molecule which encodes the structural region of hepatitis C virus has been replaced by the structural region from the genome of another hepatitis C virus strain.

7. The nucleic acid molecule of claim 6, wherein said molecule encodes the amino acid sequence shown in Figures 16G-16H.

8. The nucleic acid molecule of claim 7, wherein said molecule comprises the nucleic acid sequence shown in Figures 16A-16F.

9. The nucleic acid molecule of claim 1, wherein a fragment of the nucleic acid molecule which encodes at least one HCV protein has been replaced by a fragment of the genome of another hepatitis C virus strain which encodes the corresponding protein.

10. The nucleic acid molecule of claim 9, wherein the protein is selected from the group consisting of E1, E2 or NS4 proteins.

11. The nucleic acid molecule of claim 1, wherein a fragment of the molecule encoding all or part of an HCV protein has been deleted.

12. The nucleic acid molecule of claim 11, wherein the HCV protein is selected from the group consisting of P7, NS4B or NS5A proteins.

13. A DNA construct comprising a nucleic acid molecule according to claims 1, 3, 5 or 8.

14. An RNA transcript of the DNA construct of claim 13.

15. A cell transfected with the DNA construct of claim 13.

16. A cell transfected with RNA transcript of claim 14.

17. A hepatitis C virus polypeptide produced by the cell of claim 15.

18. A hepatitis C virus polypeptide produced by the cell of claim 16.

19. A hepatitis C virus produced by the cell of claim 13.

20. A hepatitis C virus produced by the cell of claim 14.

21. A hepatitis C virus whose genome comprises a nucleic acid molecule according to claims 1, 3, 5, 6, 8, or 9.

22. A method for producing a hepatitis C virus comprising transfecting a host cell with the RNA transcript of claim 14.

23. A polypeptide encoded by a nucleic acid sequence according to claims 1, 2, 4 or 7 or a fragment thereof.

24. The polypeptide of claim 23, wherein said polypeptide is selected from the group consisting of NS3 protease, E1 protein, E2 protein or NS4 protein.

25. A method for assaying candidate antiviral agents for activity against HCV, comprising

a) exposing a cell containing the hepatitis C virus of claim 21 to the candidate antiviral agent; and

b) measuring the presence or absence of hepatitis C virus replication in the cell of step (a).

26. The method of claim 25, wherein said replication in step (b) is measured by at least one of the following: negative strand RT-PCR, quantitative RT-PCR, Western blot, immunofluoresence, or infectivity in a susceptible animal.

27. A method for assaying candidate antiviral agents for activity against HCV, comprising:

a) exposing an HCV protease encoded by a nucleic acid sequence according to claims 1, 2, 4, or 7, or a fragment thereof to the candidate antiviral agent in the presence of a protease substrate; and

b) measuring the protease activity of said protease.

28. The method of claim 27, wherein said HCV protease is selected from the group consisting of an NS3 domain protease, an NS3-NS4A fusion polypeptide, or an NS2-NS3 protease.

29. An antiviral agent identified as having antiviral activity for HCV by the method of claim 25.

30. An antiviral agent identified as having antiviral activity for HCV by the method of claim 27.

31. Antibody to the polypeptide of claim 23.

32. Antibody to the hepatitis C virus of claim 21.

33. A method for determining the susceptibility of cells *in vitro* to support HCV infection, comprising the steps of:

a. growing animal cells in vitro;

b. transfecting into said cells the nucleic

acid of claim 1; and

c. determining if said cells show indicia of HCV replication.

34. The method according to claim 33, wherein said cells are human cells.

35. A cassette vector for cloning viral genomes, comprising, inserted therein, the nucleic acid sequence according to claim 2, said vector reading in the correct phase for the expression of said inserted sequence and having an active promoter sequence upstream thereof.

36. The cassette vector of claim 35, wherein the cassette vector is produced from plasmid pCV.

37. The cassette vector of claim 35, wherein the vector also contains one or more expressible marker genes.

38. The cassette vector of claim 35, wherein the inserted DNA sequence contains at least one ORF of the HCV genome from any strain.

39. The cassette vector of claim 35, wherein the promoter is a bacterial promoter.

40. A composition comprising a polypeptide of claim 23 suspended in a suitable amount of a pharmaceutically acceptable diluent or excipient.

41. A method for treating hepatitis C viral infection comprising the administration to a animal in need thereof of a clinically effective amount of the composition of claim 40.

42. A composition comprising a nucleic acid molecule of claim 1 suspended in a suitable amount of a pharmaceutically acceptable diluent or excipient.

43. A method for treating hepatitis C viral infection comprising the administration to an animal in need thereof of a clinically effective amount of the composition of claim 42.

ABSTRACT OF THE DISCLOSURE

The present invention discloses nucleic acid sequences which encode infectious hepatitis C viruses and the use of these sequences, and polypeptides encoded by all or part of these sequences, in the development of vaccines and diagnostics for HCV and in the development of screening assays for the identification of antiviral agents for HCV.

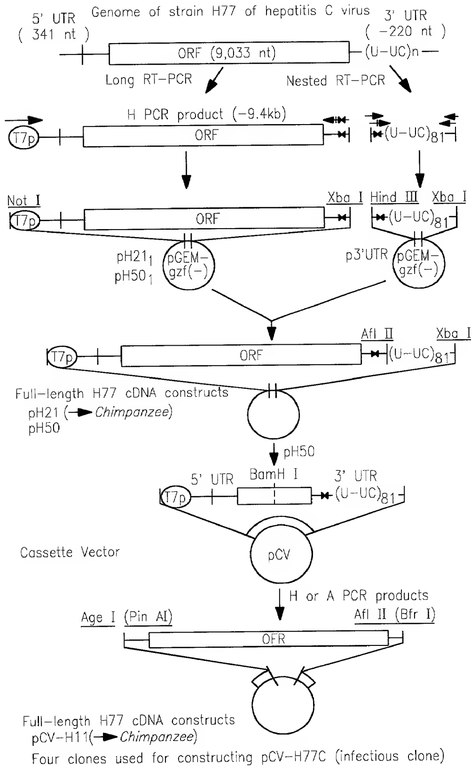

5' UTR
(341 nt)
Genome of strain H77 of hepatitis C virus
3' UTR
(-220 nt)
ORF (9,033 nt)
(U-UC)n
Long RT-PCR
Nested RT-PCR
H PCR product (-9.4kb)
T7p
ORF
(U-UC)81
Not I
Xba I
Hind III
Xba I
pH21
pH50
pGEM-gzf(-)
p3'UTR
Afl II
Full-length H77 cDNA constructs
pH21 (→ Chimpanzee)
pH50
5' UTR
BamH I
3' UTR
Cassette Vector
pCV
H or A PCR products
Age I (Pin AI)
Afl II (Bfr I)
OFR
Full-length H77 cDNA constructs
pCV-H11(→ Chimpanzee)
Four clones used for constructing pCV-H77C (infectious clone)

FIG. 1

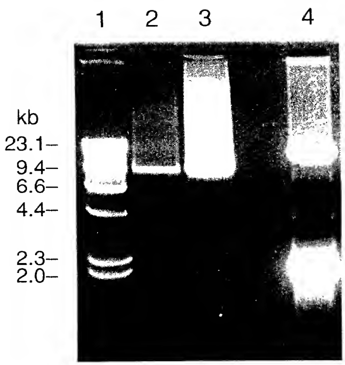


FIG. 2

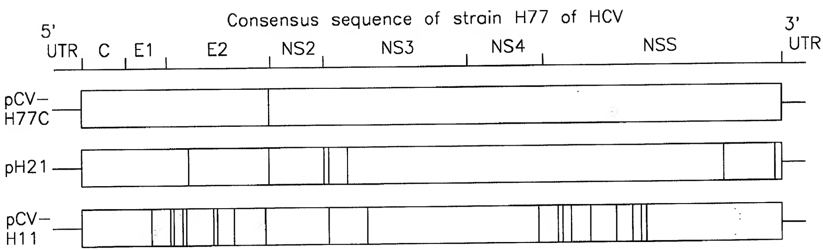

Consensus sequence of strain H77 of HCV
5' UTR
C
E1
E2
NS2
NS3
NS4
NSS
3' UTR
pCV-H77C
pH21
pCV-H11

FIG. 3

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
GCCAGCCCCC	TGATGGGGGC	GACACTCCAC	CATGAATCAC	TCCCCTGTGA	50
GGAACTACTG	TCTTCACGCA	GAAAGCGTCT	AGCCATGGCG	TTAGTATGAG	100
TGTCGTGCAG	CCTCCAGGAC	CCCCCCTCCC	GGGAGAGCCA	TAGTGGTCTG	150
CGGAACCGGT	GAGTACACCG	GAATTGCCAG	GACGACCGGG	TCCTTTCTTG	200
GATAAACCCG	CTCAATGCCT	GGAGATTTGG	GCGTGCCCCC	GCAAGACTGC	250
TAGCCGAGTA	GTGTTGGGTC	GCGAAAGGCC	TTGTGGTACT	GCCTGATAGG	300
GTGCTTGCGA	GTGCCCCGGG	AGGTCTCGTA	GACCGTGCAC	CATGAGCACG	350
AATCCTAAAC	CTCAAAGAAA	AACCAAACGT	AACACCAACC	GTCGCCCACA	400
GGACGTCAAG	TTCCCGGGTG	GCGGTCAGAT	CGTTGGTGGA	GTTTACTTGT	450
TGCCGCGCAG	GGGCCCTAGA	TTGGGTGTGC	GCGCGACGAG	GAAGACTTCC	500
GAGCGGTCGC	AACCTCGAGG	TAGACGTCAG	CCTATCCCCA	AGGCACGTCG	550
GCCCGAGGGC	AGGACCTGGG	CTCAGCCCGG	GTACCCTTGG	CCCCTCTATG	600
GCAATGAGGG	TTGCGGGTGG	GCGGGATGGC	TCCTGTCTCC	CCGTGGCTCT	650
CGGCCTAGCT	GGGGCCCCAC	AGACCCCCGG	CGTAGGTCGC	GCAATTTGGG	700
TAAGGTCATC	GATACCCTTA	CGTGCGGCTT	CGCCGACCTC	ATGGGGTACA	750
TACCGCTCGT	CGGCGCCCCT	CTTGGAGGCG	CTGCCAGGGC	CCTGGCGCAT	800
GGCGTCCGGG	TTCTGGAAGA	CGGCGTGAAC	TATGCAACAG	GGAACCTTCC	850
TGGTTGCTCT	TTCTCTATCT	TCCTTCTGGC	CCTGCTCTCT	TGCCTGACTG	900
TGCCCGCTTC	AGCCTACCAA	GTGCGCAATT	CCTCGGGGCT	TTACCATGTC	950
ACCAATGATT	GCCCTAACTC	GAGTATTGTG	TACGAGGCGG	CCGATGCCAT	1000
CCTGCACACT	CCGGGGTGTG	TCCCTTGCGT	TCGCGAGGGT	AACGCCTCGA	1050
GGTGTTGGGT	GGCGGTGACC	CCCACGGTGG	CCACCAGGGA	CGGCAAACTC	1100
CCCACAACGC	AGCTTCGACG	TCATATCGAT	CTGCTTGTCG	GGAGCGCCAC	1150
CCTCTGCTCG	GCCCTCTACG	TGGGGGACCT	GTGCGGGTCT	GTCTTTCTTG	1200
TTGGTCAACT	GTTTACCTTC	TCTCCCAGGC	GCCACTGGAC	GACGCAAGAC	1250
TGCAATTGTT	CTATCTATCC	CGGCCATATA	ACGGGTCATC	GCATGGCATG	1300
GGATATGATG	ATGAACTGGT	CCCCTACGGC	AGCGTTGGTG	GTAGCTCAGC	1350
TGCTCCGGAT	CCCACAAGCC	ATCATGGACA	TGATCGCTGG	TGCTCACTGG	1400
GGAGTCCTGG	CGGGCATAGC	GTATTTCTCC	ATGGTGGGGA	ACTGGGCGAA	1450
GGTCCTGGTA	GTGCTGCTGC	TATTTGCCGG	CGTCGACGCG	GAAACCCACG	1500
TCACCGGGGG	AAATGCCGGC	CGCACCACGG	CTGGGCTTGT	TGGTCTCCTT	1550
ACACCAGGCG	CCAAGCAGAA	CATCCAACTG	ATCAACACCA	ACGGCAGTTG	1600
GCACATCAAT	AGCACGGCCT	TGAATTGCAA	TGAAAGCCTT	AACACCGGCT	1650
GGTTAGCAGG	GCTCTTCTAT	CAACACAAAT	TCAACTCTTC	AGGCTGTCCT	1700
GAGAGGTTGG	CCAGCTGCCG	ACGCCTTACC	GATTTTGCCC	AGGGCTGGGG	1750
TCCTATCAGT	TATGCCAACG	GAAGCGGCCT	CGACGAACGC	CCCTACTGCT	1800
GGCACTACCC	TCCAAGACCT	TGTGGCATTG	TGCCCGCAAA	GAGCGTGTGT	1850
GGCCCGGTAT	ATTGCTTCAC	TCCCAGCCCC	GTGGTGGTGG	GAACGACCGA	1900

FIG. 4A

H77C

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
CAGGTCGGGC	GCGCCTACCT	ACAGCTGGGG	TGCAAATGAT	ACGGATGTCT	1950
TCGTCCTTAA	CAACACCAGG	CCACCGCTGG	GCAATTGGTT	CGGTTGTACC	2000
TGGATGAACT	CAACTGGATT	CACCAAAGTG	TGCGGAGCGC	CCCCTTGTGT	2050
CATCGGAGGG	GTGGGCAACA	ACACCTTGCT	CTGCCCCACT	GATTGCTTCC	2100
GCAAACATCC	GGAAGCCACA	TACTCTCGGT	GCGGCTCCGG	TCCCTGGATT	2150
ACACCCAGGT	GCATGGTCGA	CTACCCGTAT	AGGCTTTGGC	ACTATCCTTG	2200
TACCATCAAT	TACACCATAT	TCAAAGTCAG	GATGTACGTG	GGAGGGGTCG	2250
AGCACAGGCT	GGAAGCGGCC	TGCAACTGGA	CGCGGGGCGA	ACGCTGTGAT	2300
CTGGAAGACA	GGGACAGGTC	CGAGCTCAGC	CCGTTGCTGC	TGTCCACCAC	2350
ACAGTGGCAG	GTCCTTCCGT	GTTCTTTCAC	GACCCTGCCA	GCCTTGTCCA	2400
CCGGCCTCAT	CCACCTCCAC	CAGAACATTG	TGGACGTGCA	GTACTTGTAC	2450
GGGGTAGGGT	CAAGCATCGC	GTCCTGGGCC	ATTAAGTGGG	AGTACGTCGT	2500
TCTCCTGTTC	CTTCTGCTTG	CAGACGCGCG	CGTCTGCTCC	TGCTTGTGGA	2550
TGATGTTACT	CATATCCCAA	GCGGAGGCGG	CTTTGGAGAA	CCTCGTAATA	2600
CTCAATGCAG	CATCCCTGGC	CGGGACGCAC	GGTCTTGTGT	CCTTCCTCGT	2650
GTTCTTCTGC	TTTGCGTGGT	ATCTGAAGGG	TAGGTGGGTG	CCCCGGAGCGG	2700
TCTACGCCCT	CTACGGGATG	TGGCCTCTCC	TCCTGCTCCT	GCTGGCGTTG	2750
CCTCAGCGGG	CATACGCACT	GGACACGGAG	GTGGCCGCGT	CGTGTGGCGG	2800
CGTTGTTCTT	GTCGGGTTAA	TGGCGCTGAC	TCTGTCGCCA	TATTACAAGC	2850
GCTATATCAG	CTGGTGCATG	TGGTGGCTTC	AGTATTTTCT	GACCAGAGTA	2900
GAAGCGCAAC	TGCACGTGTG	GGTTCCCCCC	CTCAACGTCC	GGGGGGGGCG	2950
CGATGCCGTC	ATCTTACTCA	TGTGTGTAGT	ACACCCGACC	CTGGTATTTG	3000
ACATCACCAA	ACTACTCCTG	GCCATCTTCG	GACCCCTTTG	GATTCTTCAA	3050
GCCAGTTTGC	TTAAAGTCCC	CTACTTCGTG	CGCGTTCAAG	GCCTTCTCCG	3100
GATCTGCGCG	CTAGCGCGGA	AGATAGCCGG	AGGTCATTAC	GTGCAAATGG	3150
CCATCATCAA	GTTAGGGGCG	CTTACTGGCA	CCTATGTGTA	TAACCATCTC	3200
ACCCCTCTTC	GAGACTGGGC	GCACAACGGC	CTGCGAGATC	TGGCCGTGGC	3250
TGTGGAACCA	GTCGTCTTCT	CCCGAATGGA	GACCAAGCTC	ATCACGTGGG	3300
GGGCAGATAC	CGCCGCGTGC	GGTGACATCA	TCAACGGCTT	GCCCGTCTCT	3350
GCCCGTAGGG	GCCAGGAGAT	ACTGCTTGGG	CCAGCCGACG	GAATGGTCTC	3400
CAAGGGGTGG	AGGTTGCTGG	CGCCCATCAC	GGCGTACGCC	CAGCAGACGA	3450
GAGGCCTCCT	AGGGTGTATA	ATCACCAGCC	TGACTGGCCG	GGACAAAAAC	3500
CAAGTGGAGG	GTGAGGTCCA	GATCGTGTCA	ACTGCTACCC	AAACCTTCCT	3550
GGCAACGTGC	ATCAATGGGG	TATGCTGGAC	TGTCTACCAC	GGGGCCGGAA	3600
CGAGGACCAT	CGCATCACCC	AAGGGTCCTG	TCATCCAGAT	GTATACCAAT	3650
GTGGACCAAG	ACCTTGTGGG	CTGGCCCGCT	CCTCAAGGTT	CCCGCTCATT	3700
GACACCCTGT	ACCTGCGGCT	CCTCGGACCT	TTACCTGGTC	ACGAGGCACG	3750
CCGATGTCAT	TCCCGTGCGC	CGGCGAGGTG	ATAGCAGGGG	TAGCCTGCTT	3800

FIG. 4B

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TCGCCCCGGC	CCATTTCCTA	CTTGAAAGGC	TCCTCGGGGG	GTCCGCTGTT	3850
GTGCCCCGCG	GGACACGCCG	TGGGCCTATT	CAGGGCCGCG	GTGTGCACCC	3900
GTGGAGTGGC	TAAAGCGGTG	GACTTTATCC	CTGTGGAGAA	CCTAGGGACA	3950
ACCATGAGAT	CCCCGGTGTT	CACGGACAAC	TCCTCTCCAC	CAGCAGTGCC	4000
CCAGAGCTTC	CAGGTGGCCC	ACCTGCATGC	TCCCACCGGC	AGCGGTAAGA	4050
GCACCAAGGT	CCCGGCTGCG	TACGCAGCCC	AGGGCTACAA	GGTGTTGGTG	4100
CTCAACCCCT	CTGTTGCTGC	AACGCTGGGC	TTTGGTGCTT	ACATGTCCAA	4150
GGCCCATGGG	GTTGATCCTA	ATATCAGGAC	CGGGGTGAGA	ACAATTACCA	4200
CTGGCAGCCC	CATCACGTAC	TCCACCTACG	GCAAGTTCCT	TGCCGACGGC	4250
GGGTGCTCAG	GAGGTGCTTA	TGACATAATA	ATTTGTGACG	AGTGCCACTC	4300
CACGGATGCC	ACATCCATCT	TGGGCATCGG	CACTGTCCTT	GACCAAGCAG	4350
AGACTGCGGG	GGCGAGACTG	GTTGTGCTCG	CCACTGCTAC	CCCTCCGGGC	4400
TCCGTCACTG	TGTCCCATCC	TAACATCGAG	GAGGTTGCTC	TGTCCACCAC	4450
CGGAGAGATC	CCCTTTTACG	GCAAGGCTAT	CCCCCTCGAG	GTGATCAAGG	4500
GGGGAAGACA	TCTCATCTTC	TGCCACTCAA	AGAAGAAGTG	CGACGAGCTC	4550
GCCGCGAAGC	TGGTCGCATT	GGGCATCAAT	GCCGTGGCCT	ACTACCGCGG	4600
TCTTGACGTG	TCTGTCATCC	CGACCAGCGG	CGATGTTGTC	GTCGTGTCGA	4650
CCGATGCTCT	CATGACTGGC	TTTACCGGCG	ACTTCGACTC	TGTGATAGAC	4700
TGCAACACGT	GTGTCACTCA	GACAGTCGAT	TTCAGCCTTG	ACCCTACCTT	4750
TACCATTGAG	ACAACCACGC	TCCCCCAGGA	TGCTGTCTCC	AGGACTCAAC	4800
GCCGGGGCAG	GACTGGCAGG	GGGAAGCCAG	GCATCTATAG	ATTTGTGGCA	4850
CCGGGGGAGC	GCCCCTCCGG	CATGTTCGAC	TCGTCCGTCC	TCTGTGAGTG	4900
CTATGACGCG	GGCTGTGCTT	GGTATGAGCT	CACGCCCGCC	GAGACTACAG	4950
TTAGGCTACG	AGCGTACATG	AACACCCCGG	GGCTTCCCGT	GTGCCAGGAC	5000
CATCTTGAAT	TTTGGGAGGG	CGTCTTTACG	GGCCTCACTC	ATATAGATGC	5050
CCACTTTTTA	TCCCAGACAA	AGCAGAGTGG	GGAGAACTTT	CCTTACCTGG	5100
TAGCGTACCA	AGCCACCGTG	TGCGCTAGGG	CTCAAGCCCC	TCCCCCATCG	5150
TGGGACCAGA	TGTGGAAGTG	TTTGATCCGC	CTTAAACCCA	CCCTCCATGG	5200
GCCAACACCC	CTGCTATACA	GACTGGGCGC	TGTTCAGAAT	GAAGTCACCC	5250
TGACGCACCC	AATCACCAAA	TACATCATGA	CATGCATGTC	GGCCGACCTG	5300
GAGGTCGTCA	CGAGCACCTG	GGTGCTCGTT	GGCGGCGTCC	TGGCTGCTCT	5350
GGCCGCGTAT	TGCCTGTCAA	CAGGCTGCGT	GGTCATAGTG	GGCAGGATCG	5400
TCTTGTCCGG	GAAGCCGGCA	ATTATACCTG	ACAGGGAGGT	TCTCTACCAG	5450
GAGTTCGATG	AGATGGAAGA	GTGCTCTCAG	CACTTACCGT	ACATCGAGCA	5500
AGGGATGATG	CTCGCTGAGC	AGTTCAAGCA	GAAGGCCCTC	GGCCTCCTGC	5550
AGACCGCGTC	CCGCCATGCA	GAGGTTATCA	CCCCTGCTGT	CCAGACCAAC	5600
TGGCAGAAAC	TCGAGGTCTT	TTGGGCGAAG	CACATGTGGA	ATTTCATCAG	5650
TGGGATACAA	TACTTGGCGG	GCCTGTCAAC	GCTGCCTGGT	AACCCCGCCA	5700

FIG. 4C

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TTGCTTCATT	GATGGCTTTT	ACAGCTGCCG	TCACCAGCCC	ACTAACCACT	5750
GGCCAAACCC	TCCTCTTCAA	CATATTGGGG	GGGTGGGTGG	CTGCCCAGCT	5800
CGCCGCCCCC	GGTGCCGCTA	CTGCCTTTGT	GGGTGCTGGC	CTAGCTGGCG	5850
CCGCCATCGG	CAGCGTTGGA	CTGGGGAAGG	TCCTCGTGGA	CATTCTTGCA	5900
GGGTATGGCG	CGGGCGTGGC	GGGAGCTCTT	GTAGCATTCA	AGATCATGAG	5950
CGGTGAGGTC	CCCTCCACGG	AGGACCTGGT	CAATCTGCTG	CCCGCCATCC	6000
TCTCGCCTGG	AGCCCTTGTA	GTCGGTGTGG	TCTGCGCAGC	AATACTGCGC	6050
CGGCACGTTG	GCCCGGGCGA	GGGGGCAGTG	CAATGGATGA	ACCGGCTAAT	6100
AGCCTTCGCC	TCCCGGGGGA	ACCATGTTTC	CCCCACGCAC	TACGTGCCGG	6150
AGAGCGATGC	AGCCGCCCGC	GTCACTGCCA	TACTCAGCAG	CCTCACTGTA	6200
ACCCAGCTCC	TGAGGCGACT	GCATCAGTGG	ATAAGCTCGG	AGTGTACCAC	6250
TCCATGCTCC	GGTTCCTGGC	TAAGGGACAT	CTGGGACTGG	ATATGCGAGG	6300
TGCTGAGCGA	CTTTAAGACC	TGGCTGAAAG	CCAAGCTCAT	GCCACAACTG	6350
CCTGGGATTC	CCTTTGTGTC	CTGCCAGCGC	GGGTATAGGG	GGGTCTGGCG	6400
AGGAGACGGC	ATTATGCACA	CTCGCTGCCA	CTGTGGAGCT	GAGATCACTG	6900
GACATGTCAA	AAACGGGACG	ATGAGGATCG	TCGGTCCTAG	GACCTGCAGG	6950
AACATGTGGA	GTGGGACGTT	CCCCATTAAC	GCCTACACCA	CGGGCCCCTG	6550
TACTCCCCTT	CCTGCGCCGA	ACTATAAGTT	CGCGCTGTGG	AGGGTGTCTG	6600
CAGAGGAATA	CGTGGAGATA	AGGCGGGTGG	GGGACTTCCA	CTACGTATCG	6650
GGTATGACTA	CTGACAATCT	TAAATGCCCG	TGCCAGATCC	CATCGCCCGA	6700
ATTTTTCACA	GAATTGGACG	GGGTGCGCCT	ACACAGGTTT	GCGCCCCCTT	6750
GCAAGCCCTT	GCTGCGGGAG	GAGGTATCAT	TCAGAGTAGG	ACTCCACGAG	6800
TACCCGGTGG	GGTCGCAATT	ACCTTGCGAG	CCCGAACCGG	ACGTAGCCGT	6850
GTTGACGTCC	ATGCTCACTG	ATCCCTCCCA	TATAACAGCA	GAGGCGGCCG	6900
GGAGAAGGTT	GGCGAGAGGG	TCACCCCCTT	CTATGGCCAG	CTCCTCGGCT	6950
AGCCAGCTGT	CCGCTCCATC	TCTCAAGGCA	ACTTGCACCG	CCAACCATGA	7000
CTCCCCTGAC	GCCGAGCTCA	TAGAGGCTAA	CCTCCTGTGG	AGGCAGGAGA	7050
TGGGCGGCAA	CATCACCAGG	GTTGAGTCAG	AGAACAAAGT	GGTGATTCTG	7100
GACTCCTTCG	ATCCGCTTGT	GGCAGAGGAG	GATGAGCGGG	AGGTCTCCGT	7150
ACCTGCAGAA	ATTCTGCGGA	AGTCTCGGAG	ATTCGCCCGG	GCCCTGCCCG	7200
TCTGGGCGCG	GCCGGACTAC	AACCCCCCGC	TAGTAGAGAC	GTGGAAAAAG	7250
CCTGACTACG	AACCACCTGT	GGTCCATGGC	TGCCCGCTAC	CACCTCCACG	7300
GTCCCCTCCT	GTGCCTCCGC	CTCGGAAAAA	GCGTACGGTG	GTCCTCACCG	7350
AATCAACCCT	ATCTACTGCC	TTGGCCGAGC	TTGCCACCAA	AAGTTTTGGC	7400
AGCTCCTCAA	CTTCCGGCAT	TACGGGCGAC	AATACGACAA	CATCCTCTGA	7450
GCCCGCCCCT	TCTGGCTGCC	CCCCCGACTC	CGACGTTGAG	TCCTATTCTT	7500
CCATGCCCCC	CCTGGAGGGG	GAGCCTGGGG	ATCCGGATCT	CAGCGACGGG	7550
TCATGGTCGA	CGGTCAGTAG	TGGGGCCGAC	ACGGAAGATG	TCGTGTGCTG	7600

FIG. 4D

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
CTCAATGTCT	TATTCCTGGA	CAGGCGCACT	CGTCACCCCG	TGCGCTGCGG	7650
AAGAACAAAA	ACTGCCCATC	AACGCACTGA	GCAACTCGTT	GCTACGCCAT	7700
CACAATCTGG	TGTATTCCAC	CACTTCACGC	AGTGCTTGCC	AAAGGCAGAA	7750
GAAAGTCACA	TTTGACAGAC	TGCAAGTTCT	GGACAGCCAT	TACCAGGACG	7800
TGCTCAAGGA	GGTCAAAGCA	GCGGCGTCAA	AAGTGAAGGC	TAACTTGCTA	7850
TCCGTAGAGG	AAGCTTGCAG	CCTGACGCCC	CCACATTCAG	CCAAATCCAA	7900
GTTTGGCTAT	GGGGCAAAAG	ACGTCCGTTG	CCATCCCAGA	AAGGCCGTAG	7950
CCCACATCAA	CTCCGTGTGG	AAAGACCTTC	TGGAAGACAG	TGTAACACCA	8000
ATAGACACTA	CCATCATGGC	CAAGAACGAG	GTTTTCTGCG	TTCAGCCTGA	8050
GAAGGGGGGT	CGTAAGCCAG	CTCGTCTCAT	CGTGTTCCCC	GACCTGGGCG	8100
TGCGCGTGTG	CGAGAAGATG	GCCCTGTACG	ACGTGGTTAG	CAAGCTCCCC	8150
CTGGCCGTGA	TGGGAAGCTC	CTACGGATTC	CAATACTCAC	CAGGACAGCG	8200
GGTTGAATTC	CTCGTGCAAG	CGTGGAAGTC	CAAGAAGACC	CCGATGGGGT	8250
TCTCGTATGA	TACCCGCTGT	TTTGACTCCA	CAGTCACTGA	GAGCGACATC	8300
CGTACGGAGG	AGGCAATTTA	CCAATGTTGT	GACCTGGACC	CCCAAGCCCG	8350
CGTGGCCATC	AAGTCCCTCA	CTGAGAGGCT	TTATGTTGGG	GGCCCTCTTA	8400
CCAATTCAAG	GGGGGAAAAC	TGCGGCTACC	GCAGGTGCCG	CGCGAGCGGC	8450
GTACTGACAA	CTAGCTGTGG	TAACACCCTC	ACTTGCTACA	TCAAGGCCCG	8500
GGCAGCCTGT	CGAGCCGCAG	GGCTCCAGGA	CTGCACCATG	CTCGTGTGTG	8550
GCGACGACTT	AGTCGTTATC	TGTGAAAGTG	CGGGGGTCCA	GGAGGACGCG	8600
GCGAGCCTGA	GAGCCTTCAC	GGAGGCTATG	ACCAGGTACT	CCGCCCCCCC	8650
CGGGGACCCC	CCACAACCAG	AATACGACTT	GGAGCTTATA	ACATCATGCT	8700
CCTCCAACGT	GTCAGTCGCC	CACGACGGCG	CTGGAAAGAG	GGTCTACTAC	8750
CTTACCCGTG	ACCCTACAAC	CCCCCTCGCG	AGAGCCGCGT	GGGAGACAGC	8800
AAGACACACT	CCAGTCAATT	CCTGGCTAGG	CAACATAATC	ATGTTTGCCC	8850
CCACACTGTG	GGCGAGGATG	ATACTGATGA	CCCATTTCTT	TAGCGTCCTC	8900
ATAGCCAGGG	ATCAGCTTGA	ACAGGCTCTT	AACTGTGAGA	TCTACGGAGC	8950
CTGCTACTCC	ATAGAACCAC	TGGATCTACC	TCCAATCATT	CAAAGACTCC	9000
ATGGCCTCAG	CGCATTTTCA	CTCCACAGTT	ACTCTCCAGG	TGAAATCAAT	9050
AGGGTGGCCG	CATGCCTCAG	AAAACTTGGG	GTCCCGCCCT	TGCGAGCTTG	9100
GAGACACCGG	GCCCGGAGCG	TCCGCGCTAG	GCTTCTGTCC	AGAGGAGGCA	9150
GGGCTGCCAT	ATGTGGCAAG	TACCTCTTCA	ACTGGGCAGT	AAGAACAAAG	9200
CTCAAACTCA	CTCCAATAGC	GGCCGCTGGC	CGGCTGGACT	TGTCCGGTTG	9250
GTTCACGGCT	GGCTACAGCG	GGGGAGACAT	TTATCACAGC	GTGTCTCATG	9300
CCCGGCCCCG	CTGGTTCTGG	TTTTGCCTAC	TCCTGCTCGC	TGCAGGGGTA	9350
GGCATCTACC	TCCTCCCCAA	CCGATGAAGG	TTGGGGTAAA	CACTCCGGCC	9400
TCTTAAGCCA	TTTCCTGTTT	TTTTTTTTTT	TTTTTTTTTT	TTTTTCTTTT	9450
TTTTTTTCTT	TCCTTTCCTT	CTTTTTTTCC	TTTCTTTTTC	CCTTCTTTAA	9500

FIG. 4E

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TGGTGGCTCC	ATCTTAGCCC	TAGTCACGGC	TAGCTGTGAA	AGGTCCGTGA	9550
GCCGCATGAC	TGCAGAGAGT	GCTGATACTG	GCCTCTCTGC	AGATCATGT	9599

FIG. 4F

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
MSTNPKPQRK	TKRNTNRRPQ	DVKFPGGGQI	VGGVYLLPRR	GPRLGVRATR	50
KTSERSQPRG	RRQPIPKARR	PEGRTWAQPG	YPWPLYGNEG	CGWAGWLLSP	100
RGSRPSWGPT	DPRRRSRNLG	KVIDTLTCGF	ADLMGYIPLV	GAPLGGAARA	150
LAHGVRVLED	GVNYATGNLP	GCSFSIFLLA	LLSCLTVPAS	AYQVRNSSGL	200
YHVTNDCPNS	SIVYEAADAI	LHTPGCVPCV	REGNASRCWV	AVTPTVATRD	250
GKLPTTQLRR	HIDLLVGSAT	LCSALYVGDL	CGSVFLVGQL	FTFSPRRHWT	300
TQDCNCSIYP	GHITGHRMAW	DMMMNWSPTA	ALVVAQLLRI	PQAIMDMIAG	350
AHWGVLAGIA	YFSMVGNWAK	VLVVLLLFAG	VDAETHVTGG	NAGRTTAGLV	400
GLLTPGAKQN	IQLINTNGSW	HINSTALNCN	ESLNTGWLAG	LFYQHKFNSS	450
GCPERLASCR	RLTDFAQGWG	PISYANGSGL	DERPYCWHYP	PRPCGIVPAK	500
SVCGPVYCFT	PSPVVVGTTD	RSGAPTYSWG	ANDTDVFVLN	NTRPPLGNWF	550
GCTWMNSTGF	TKVCGAPPCV	IGGVGNNTLL	CPTDCFRKHP	EATYSRCGSG	600
PWITPRCMVD	YPYRLWHYPC	TINYTIFKVR	MYVGGVEHRL	EAACNWTRGE	650
RCDLEDRDRS	ELSPLLLSTT	QWQVLPCSFT	TLPALSTGLI	HLHQNIVDVQ	700
YLYGVGSSIA	SWAIKWEYVV	LLFLLLADAR	VCSCLWMMLL	ISQAEAALEN	750
LVILNAASLA	GTHGLVSFLV	FFCFAWYLKG	RWVPGAVYAL	YGMWPLLLLL	800
LALPQRAYAL	DTEVAASCGG	VVLVGLMALT	LSPYYKRYIS	WCMWWLQYFL	850
TRVEAQLHVW	VPPLNVRGGR	DAVILLMCVV	HPTLVFDITK	LLLAIFGPLW	900
ILQASLLKVP	YFVRVQGLLR	ICALARKIAG	GHYVQMAIIK	LGALTGTYVY	950
NHLTPLRDWA	HNGLRDLAVA	VEPVVFSRME	TKLITWGADT	AACGDIINGL	1000
PVSARRGQEI	LLGPADGMVS	KGWRLLAPIT	AYAQQTRGLL	GCIITSLTGR	1050
DKNQVEGEVQ	IVSTATQTFL	ATCINGVCWT	VYHGAGTRTI	ASPKGPVIQM	1100
YTNVDQDLVG	WPAPQGSRSL	TPCTCGSSDL	YLVTRHADVI	PVRRRGDSRG	1150
SLLSPRPISY	LKGSSGGPLL	CPAGHAVGLF	RAAVCTRGVA	KAVDFIPVEN	1200
LGTTMRSPVF	TDNSSPPAVP	QSFQVAHLHA	PTGSGKSTKV	PAAYAAQGYK	1250
VLVLNPSVAA	TLGFGAYMSK	AHGVDPNIRT	GVRTITTGSP	ITYSTYGKFL	1300
ADGGCSGGAY	DIIICDECHS	TDATSILGIG	TVLDQAETAG	ARLVVLATAT	1350
PPGSVTVSHP	NIEEVALSTT	GEIPFYGKAI	PLEVIKGGRH	LIFCHSKKKC	1400
DELAAKLVAL	GINAVAYYRG	LDVSVIPTSG	DVVVVSTDAL	MTGFTGDFDS	1450
VIDCNTCVTQ	TVDFSLDPTF	TIETTTLPQD	AVSRTQRRGR	TGRGKPGIYR	1500
FVAPGERPSG	MFDSSVLCEC	YDAGCAWYEL	TPAETTVRLR	AYMNTPGLPV	1550
CQDHLEFWEG	VFTGLTHIDA	HFLSQTKQSG	ENFPYLVAYQ	ATVCARAQAP	1600
PPSWDQMWKC	LIRLKPTLHG	PTPLLYRLGA	VQNEVTLTHP	ITKYIMTCMS	1650
ADLEVVTSTW	VLVGGVLAAL	AAYCLSTGCV	VIVGRIVLSG	KPAIIPDREV	1700
LYQEFDEMEE	CSQHLPYIEQ	GMMLAEQFKQ	KALGLLQTAS	RHAEVITPAV	1750
QTNWQKLEVF	WAKHMWNFIS	GIQYLAGLST	LPGNPAIASL	MAFTAAVTSP	1800
LTTGQTLLFN	ILGGWVAAQL	AAPGAATAFV	GAGLAGAAIG	SVGLGKVLVD	1850
ILAGYGAGVA	GALVAFKIMS	GEVPSTEDLV	NLLPAILSPG	ALVVGVVCAA	1900

FIG. 4G

H77C

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
ILRRHVGPGE	GAVQWMNRLI	AFASRGNHVS	PTHYVPESDA	AARVTAILSS	1950
LTVTQLLRRL	HQWISSECTT	PCSGSWLRDI	WDWICEVLSD	FKTWLKAKLM	2000
PQLPGIPFVS	CQRGYRGVWR	GDGIMHTRCH	CGAEITGHVK	NGTMRIVGPR	2050
TCRNMWSGTF	PINAYTTGPC	TPLPAPNYKF	ALWRVSAEEY	VEIRRVGDFH	2100
YVSGMTTDNL	KCPCQIPSPE	FFTELDGVRL	HRFAPPCKPL	LREEVSFRVG	2150
LHEYPVGSQL	PCEPEPDVAV	LTSMLTDPSH	ITAEAAGRRL	ARGSPPSMAS	2200
SSASQLSAPS	LKATCTANHD	SPDAELIEAN	LLWRQEMGGN	ITRVESENKV	2250
VILDSFDPLV	AEEDEREVSV	PAEILRKSRR	FARALPVWAR	PDYNPPLVET	2300
WKKPDYEPPV	VHGCPLPPPR	SPPVPPPRKK	RTVVLTESTL	STALAELATK	2350
SFGSSSTSGI	TGDNTTTSSE	PAPSGCPPDS	DVESYSSMPP	LEGEPGDPDL	2400
SDGSWSTVSS	GADTEDVVCC	SMSYSWTGAL	VTPCAAEEQK	LPINALSNSL	2450
LRHHNLVYST	TSRSACQRQK	KVTFDRLQVL	DSHYQDVLKE	VKAAASKVKA	2500
NLLSVEEACS	LTPPHSAKSK	FGYGAKDVRC	HARKAVAHIN	SVWKDLLEDS	2550
VTPIDTTIMA	KNEVFCVQPE	KGGRKPARLI	VFPDLGVRVC	EKMALYDVVS	2600
KLPLAVMGSS	YGFQYSPGQR	VEFLVQAWKS	KKTPMGFSYD	TRCFDSTVTE	2650
SDIRTEEAIY	QCCDLDPQAR	VAIKSLTERL	YVGGPLTNSR	GENCGYRRCR	2700
ASGVLTTSCG	NTLTCYIKAR	AACRAAGLQD	CTMLVCGDDL	VVICESAGVQ	2750
EDAASLRAFT	EAMTRYSAPP	GDPPQPEYDL	ELITSCSSNV	SVAHDGAGKR	2800
VYYLTRDPTT	PLARAAWETA	RHTPVNSWLG	NIIMFAPTLW	ARMILMTHFF	2850
SVLIARDQLE	QALNCEIYGA	CYSIEPLDLP	PIIQRLHGLS	AFSLHSYSPG	2900
EINRVAACLR	KLGVPPLRAW	RHRARSVRAR	LLSRGGRAAI	CGKYLFNWAV	2950
RTKLKLTPIA	AAGRLDLSGW	FTAGYSGGDI	YHSVSHARPR	WFWFCLLLLA	3000
AGVGIYLLPN	R				3011

FIG. 4H

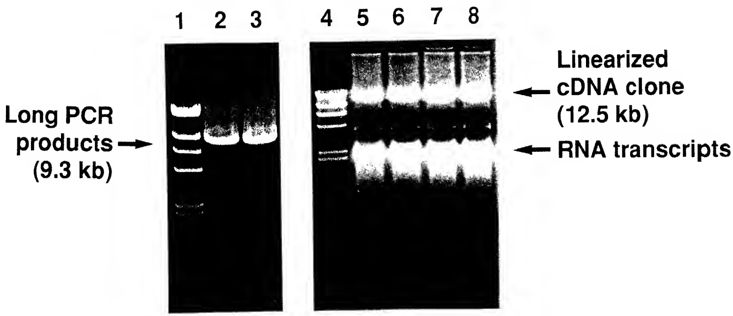

1
2
3
4
5
6
7
8
Long PCR products (9.3 kb)
Linearized cDNA clone (12.5 kb)
RNA transcripts

FIG. 5

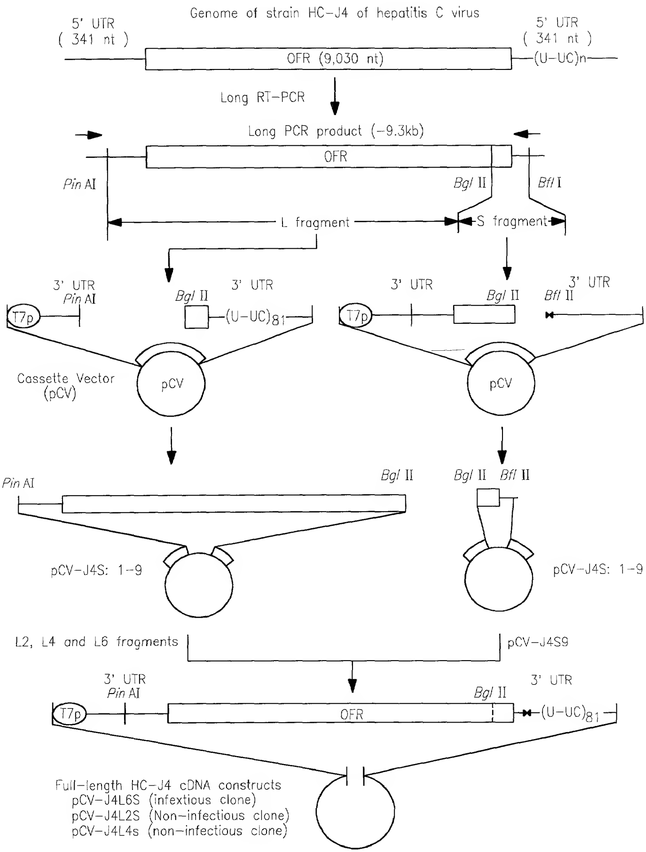

Genome of strain HC-J4 of hepatitis C virus
5' UTR
(341 nt)
OFR (9,030 nt)
5' UTR
(341 nt)
(U-UC)n
Long RT-PCR
Long PCR product (~9.3kb)
OFR
Pin AI
Bgl II
Bfl I
L fragment
S fragment
3' UTR
Pin AI
Bgl II
3' UTR
T7p
(U-UC)81
3' UTR
Bgl II
3' UTR
Bfl II
T7p
Cassette Vector
(pCV)
pCV
pCV
Pin AI
Bgl II
Bgl II
Bfl II
pCV-J4S: 1-9
pCV-J4S: 1-9
L2, L4 and L6 fragments
pCV-J4S9
3' UTR
Pin AI
Bgl II
3' UTR
T7p
OFR
(U-UC)81
Full-length HC-J4 cDNA constructs
pCV-J4L6S (infextious clone)
pCV-J4L2S (Non-infectious clone)
pCV-J4L4s (non-infectious clone)

FIG. 6

	L fragment	Cons-p9	L1* (A)	L2(A)	L6(A)	L8(A)	L9(A)	L3(B)	L7*(B)	L10(B)	L4(C)	Cons-D	Cons-F
Core	16	N	S	•	•	•	•	•	•	•	•	•	N
	36	L	•	•	•	P	•	•	•	•	•	•	L
	52	A	•	•	•	•	•	T	T	T	T	T	A,T
	70	R	•	•	•	•	•	Q	Q	Q	•	R,Q	R,Q
	189	A	•	•	•	•	•	•	T	•	•	•	A
E1	195	R	•	•	•	•	•	H	•	H	•	•	R
	231	R	•	Q	Q	Q	•	•	•	•	•	•	R
	233	G	•	•	•	•	•	A	A	A	•	•	G
	234	N	•	•	•	•	•	D	D	D	•	•	N
	250	N	•	•	•	•	•	•	•	•	•	•	N
	299	E	A	•	•	•	A	•	•	•	•	•	E
	304	C	•	•	•	•	•	•	•	•	•	•	C
	379	A	•	•	•	•	•	T	•	T	•	•	A

FIG. 7A

	L fragment	Cons-p9	L1* (A)	L2(A)	L6(A)	L8(A)	L9(A)	L3(B)	L7*(B)	L10(B)	L4(C)	Cons--D	Cons-F
E2	384	E	•	•	•	•	•	T	T	T	•	E,T	A
	386	H	•	•	•	•	•	V	V	V	•	H,Y	H,Y
	388	T	•	•	•	•	•	S	S	S	•	T,S	T,S
	390	R	•	•	•	•	•	G	G	G	•	G	R,G
	391	V	•	•	•	•	•	•	•	A	•	•	V
	392	A	V	•	•	V	V	•	•	•	V	V	A,V
	394	H	•	•	•	•	•	R	R	R	R	•	H
	405	S	•	•	•	•	•	•	P	•	•	•	S
	434	Q	•	•	•	•	•	H	H	H		H	Q,H
	438	F	•	•	•	•	•	L	L	L	L	L	F,L
	444	A	•	•	•	•	•	T	T	T		T	A,T
	450	S	•	•	•	•	•	•	•	•	P	•	S
	458	S	•	•	•	N	•	•	•	•	•	•	S
	466	A	•	•	•	•	•	V	V	V		A,V	A,V
	474	Y	•	•	•	•	•	H	•	•	•	•	Y
	476	K	•	•	•	•	•	E	E	E	E	E	K,E
	496	V	•	•	•	•	•	I	I	I	I	I	V,I
	524	V	•	•	•	•	A	•	A	•	•	•	V
	536	V	•	M	•	•	•	•	•	•	•	•	V
	580	I	•	•	•	•	•	•	V	•	•	•	I
	622	L	V	•	•	•	V	•	•	•	•	•	L
	673	Q	•	•	•	P		•	•	•	•	•	Q
p7	783	A	•	•	•	•	•	•	V	•	•	•	A

FIG. 7B

	L fragment	Cons-p9	L1* (A)	L2(A)	L6(A)	L8(A)	L9(A)	L3(B)	L7*(B)	L10(B)	L4(C)	Cons-D	Cons-F
NS2	820	G	•	•	•	•	•	•	S	•	•	•	G
	857	M	•	•	•	•	•	I		•	•	•	M
	927	K	•	•	•	•	•	•	R	•	•	•	K
	934	V	I	I	•	I	I	•	•	•	•	•	V
	937	A	•	•	V	•	•	•	•	•	•	•	A
	978	A	•	•	•	•	•	D	D	D	•	D	A,D
NS3	1028	P	•	•	•	S	•	•	•	•	•	•	P
	1031	A	•	•	•	•	•	•	T	•	•	•	A
	1043	V	•	•	I	•	I	I	I	•	I	•	V,I
	1067	Q	•	•	•	•	H	H	H	•	•	H,Q	Q,H
	1097	I	X	•	•	•	•	•	•	•	•	•	I
	1188	G	R	•	•	•	•	•	•	•	•	•	G
	1215	S	•	•	T	•	•	•	•	•	•	•	S
	1223	F	•	S	•	•	•	•	•	•	•	•	F
	1226	A	•	•	•	•	•	•	•	V	•	•	A
	1339	A	V	•	•	•	•	•	•	•	•	•	A
	1399	K	N	•	•	•	•	•	•	•	•	•	K
	1503	T	•	•	•	•	•	S	•	S	•	•	T
	1528	Y	•	•	•	•	•	•	•	•	•	•	Y
	1535	T	A	•	•	•	•	•	•	•	•	•	T
NS4A	1662	L	•	P	•	•	•	•	•	•	•	•	L

FIG. 7C

	L fragment	Cons-p9	L1* (A)	L2(A)	L6(A)	L8(A)	L9(A)	L3(B)	L7*(B)	L10(B)	L4(C)	Cons-D	Cons-F
NS4B	1753	K	•	P	•	•	•	•	•	•	•	•	K
	1805	H	•	•	N	•	•	N	•	N	N	N	H,N
	1949	S	•	•	•	•	•	•	•	•	P	•	S
NS5A	2105	M	•	•	•	•	V	I	•	I	•	•	M
	2136	K	•	•	•	•	•		•	•	R	•	K
	2146	T	•	•	•	•	•	A	A	A	•	T,A	T,A
	2226	L	•	•	•	•	•	P	•	•	•	•	L
	2259	L	•	•	•	•	•	F	•	•	•	•	L
	2262	E	•	•	•	•	•	D	D	D	•	E,D	E,D
	2334	V	•	•	•	•	•	I	•	•	•	•	V
	2371	L	•	•	•	•	•	Q	Q	Q	•	L,Q	L,Q
	2385	Y	•	•	•	•	•	•	•	•	H	•	Y
NS5B	2692	N	•	•	•	•	•	•	S	•	•	•	N
	2757	A	•	•	•	•	•	•	•	•	•	•	A
	2785	C	•	R	•	•	•	•	•	•	•	•	C
	2824	I	•	V	•	•	•	•	•	•	•	•	I
	2861	A	•	•	•	•	•	V	•	•	•	•	A
	S fragment		S5	S9	S2	S3	S7	S8	S10	S4	S6		
	2968	G	•	•	•	•	•	S	S	•	•	•	G
	2975	S	•	•	•	•	•	G	G	G	G	•	S
	2978	D	•	•	•	•	•	•	•	•	G	•	D
	2999	S	•	F	F	F	•	•	•	•	•	•	S

FIG. 7D

aa \ nt	L1 (A)	L2 (A)	L6 (A)	L8 (A)	L9 (A)	L3 (B)	L7 (B)	L10 (B)	L4 (C)	HC-J4/91	HC-J4/83
L1 (A)		0.56	0.60	0 36	0.33	1.50	1.53	1.46	0.95	0.83	1.79
L2 (A)	0.59		0.55	0.35	0.50	1.49	1.51	1.45	0.98	0.82	1.77
L6 (A)	0.52	0.42		0.31	0.55	1.33	1.38	1.29	0.80	0.68	1.58
L8 (A)	0.42	0.38	0.31		0.31	1.32	1.34	1.28	0.79	0.65	1.62
L9 (A)	0.35	0.52	0.45	0.35		1.42	1.42	1.38	0.91	0.75	1.66
L3 (B)	1.47	1.43	1.15	1.33	1.36		0.61	0.30	1.43	0.90	1.51
L7 (B)	1.36	1.33	1.05	1.22	1.22	0.66		0.57	1.47	0.95	1.54
L10 (B)	1.36	1.33	0.59	1.22	1.26	0.31	0.56		1.37	0.85	1.42
L4 (C)	0.77	0.80	0.59	0.63	1.26	1.12	1.08	1.01		0.76	1.73
HC-J4/91	0.94	0.91	0.63	0.80	0.87	0.77	0.73	0 66	0.52		1.22
HC-J4/83	1.96	1.89	1.68	1.85	1.82	1.75	1.61	1.61	1.71	1.40	

FIG. 8

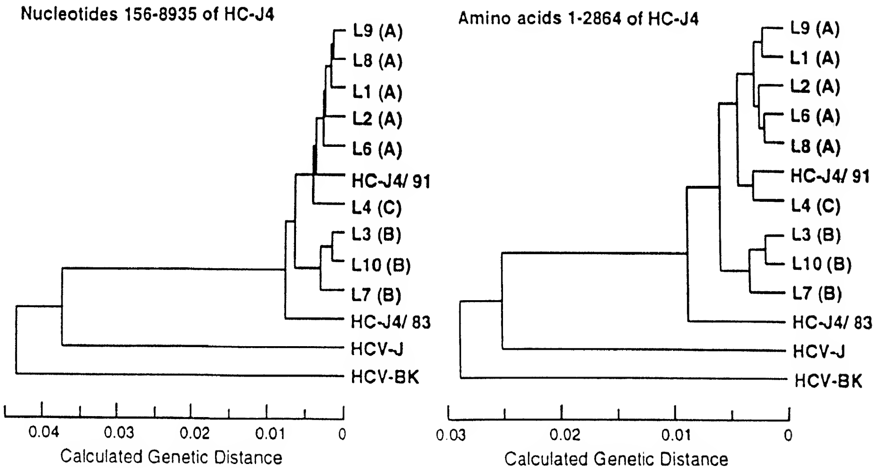

Nucleotides 156-8935 of HC-J4
L9 (A)
L8 (A)
L1 (A)
L2 (A)
L6 (A)
HC-J4/ 91
L4 (C)
L3 (B)
L10 (B)
L7 (B)
HC-J4/ 83
HCV-J
HCV-BK
0.04
0.03
0.02
0.01
0
Calculated Genetic Distance
Amino acids 1-2864 of HC-J4
L9 (A)
L1 (A)
L2 (A)
L6 (A)
L8 (A)
HC-J4/ 91
L4 (C)
L3 (B)
L10 (B)
L7 (B)
HC-J4/ 83
HCV-J
HCV-BK
0.03
0.02
0.01
0
Calculated Genetic Distance

FIG. 9

```
                      379                                 413     468                    486
HC-J4L6  (A)   :  AGVDG ETHTTGRVAGHTTSGFTSLFSSGAS QKIQL   GWGPIT YTKPNSS DQRPYC
HC-J4L2  (A)   :  ..... ......................... .....   ...... ....... ......
HC-J4/91-20    :  ..... ..............R.......... .....   ...... ..E.... ......
HC-J4L1  (A)   :  ..... ........V................ .....   ...... ....... ......
HC-J4L8  (A)   :  ..... ........V................ .....   ...... ....... ......
HC-J4L9  (A)   :  ..... ........V................ .....   ...... ....... ......
HC-J4/91-21    :  ..... ........V................ .....   ...... ..G.... ......

HC-J4L4  (C)   :  ..... ........V.R.............. .....   ...... ..E.... ......
HC-J4/91-23    :  ..... ........V.R.............. .....   ...... ..E.... ......
HC-J4/91-22    :  ..... ........V.R.............. .....   .....A ..E.... ......

HC-J4L7  (B)   :  ..... T.Y.S.G...R..........P... .....   ...... ..E.... ......
HC-J4L10(B)    :  T.... T.Y.S.GA..R.............. .....   ...... ..E.... ......
HC-J4L3  (B)   :  T.... T.Y.S.G...R.............. .....   ...... H.E.... ......
HC-J4/91-26    :  T.... T.Y.S.G...R.............. .....   ...... ..G.D.L ......
HC-J4/91-25    :  ..... A.Y.S.G...R.............. .....   ...... ..E.... ......
HC-J4/91-24    :  ..... A.Y.S.G...R.............. .....   ...... ..E...P ......
HC-J4/91       :  ..... A.Y.S.G...R.............. .....   ...... ..E...P ......

HC-J4/91-27    :  ..... K.Y.S.GA.S....R......P... .R...   ...... ..ESG.R ......
HC-J4/83       :  ..... ..Y.S.GA.S....TLA....P... .R...   ...... ..E.D.P ......
                        <---------------------->                 <----->
                                  HVR1                            HVR2
```

FIG. 10

5' Untranslated Region

```
             1                                                                                                    90
HC-J4     :GCCAGCCCCC GATTGGGGGC GACACTCCAC CATAGATCAC TCCCCTGTGA GGAACTACTG TCTTCACGCA GAAAGCGTCT AGCCATGGCG
pCV-J4L6S:.......... TGA....... .......... ...GA..... .......... .......... .......... .......... ..........
pCV-H77C :.......... TGA....... .......... ...GA..... .......... .......... .......... .......... ..........

             91                                                                                                  180
HC-J4     :TTAGTATGAG TGTCGTGCAG CCTCCAGGAC CCCCCCTCCC GGGAGAGCCA TAGTGGTCTG CGGAACCGGT GAGTACACCG GAATTGCCAG
pCV-J4L6S:.......... .......... .......... .......... .......... .......... .......... .......... ..........
pCV-H77C :.......... .......... .......... .......... .......... .......... .......... .......... ..........
                                                                                 Pin Al

             181                                                                                                 270
HC-J4     :GACGACCGGG TCCTTTCTTG GATCAACCCG CTCAATGCCT GGAGATTTGG GCGTGCCCCC GCGAGACTGC TAGCCGAGTA GTGTTGGGTC
pCV-J4L6S:.......... .......... .......... .......... .......... .......... .......... .......... ..........
pCV-H77C :.......... .......... ...A...... .......... .......... .......... ..A....... .......... ..........

             271                                                                        341
HC-J4     :GCGAAAGGCC TTGTGGTACT GCCTGATAGG GTGCTTGCGA GTGCCCCGGG AGGTCTCGTA GACCGTGCAC C
pCV-J4L6S:.......... .......... .......... .......... .......... .......... .......... .
pCV-H77C :.......... .......... .......... .......... .......... .......... .......... .
```

3' Untranslated Region

```
                          3' variable region                      poly U-UC region          3' variable region
             9372                                                                                       9513
HC-J4     :TGAACGGGGA GCTAACCACT CCAGGCCAAT AGGCCTT--C  CTG    poly (U-UC)n      ---GGTGGCT CCATCTTAG
pCV-J4L6S:.......... .......... .......-T. .A...A.TT.  ...    poly (U-UC)81     AAT....... .........
pCV-H77C :....G.TT.G .G...A.... ..G.C.TCT. .A...A.TT.  ...    poly (U-UC)81     AAT....... .........
                                         Bfr 1

                                          3' conserved region (Cont.)
             9514                                                                                      9595
H77       :CCCTAGTCAC GGCTAGCTGT GAAAGGTCCG TGAGCCGCAT GACTGCAGAG AGTGCTGATA CTGGCCTCTC TGCAGATCAT GT
pCV-J4L6S:.......... .......... .......... .......... .......... .......... .......... .......... ..
pCV-H77C :.......... .......... .......... .......... .......... .......... .......... .......... ..
```

FIG. 11

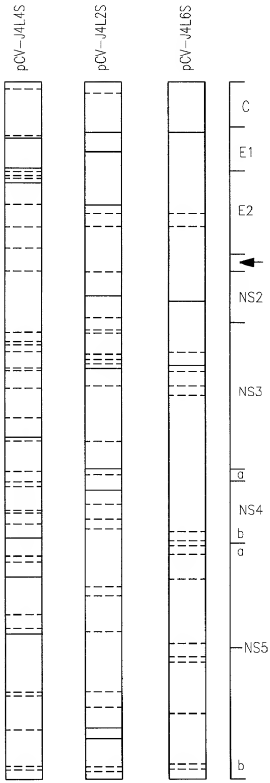


FIG. 12

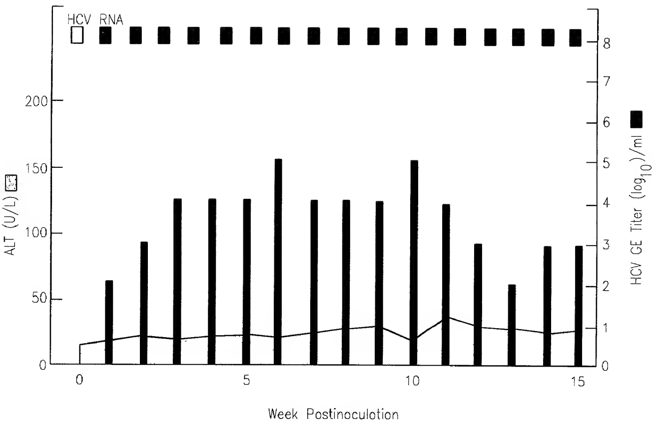

HCV RNA
ALT (U/L)
HCV GE Titer (log10)/ml
Week Postinoculotion
0
50
100
150
200
0
1
2
3
4
5
6
7
8
0
5
10
15

FIG. 13

HC-J4

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
GCCAGCCCCC	TGATGGGGGC	GACACTCCAC	CATGAATCAC	TCCCCTGTGA	50
GGAACTACTG	TCTTCACGCA	GAAAGCGTCT	AGCCATGGCG	TTAGTATGAG	100
TGTCGTGCAG	CCTCCAGGAC	CCCCCCTCCC	GGGAGAGCCA	TAGTGGTCTG	150
CGGAACCGGT	GAGTACACCG	GAATTGCCAG	GACGACCGGG	TCCTTTCTTG	200
GATCAACCCG	CTCAATGCCT	GGAGATTTGG	GCGTGCCCCC	GCGAGACTGC	250
TAGCCGAGTA	GTGTTGGGTC	GCGAAAGGCC	TTGTGGTACT	GCCTGATAGG	300
GTGCTTGCGA	GTGCCCCGGG	AGGTCTCGTA	GACCGTGCAC	CATGAGCACG	350
AATCCTAAAC	CTCAAAGAAA	AACCAAACGT	AACACCAACC	GCCGCCCACA	400
GGACGTCAAG	TTCCCGGGCG	GTGGTCAGAT	CGTTGGTGGA	GTTTACCTGT	450
TGCCGCGCAG	GGGCCCCAGG	TTGGGTGTGC	GCGCGACTAG	GAAGGCTTCC	500
GAGCGGTCGC	AACCTCGTGG	AAGGCGACAA	CCTATCCCAA	AGGCTCGCCG	550
ACCCGAGGGC	AGGGCCTGGG	CTCAGCCCGG	GTACCCTTGG	CCCCTCTATG	600
GCAATGAGGG	CCTGGGGTGG	GCAGGATGGC	TCCTGTCACC	CCGCGGCTCC	650
CGGCCTAGTT	GGGGCCCCAC	GGACCCCCGG	CGTAGGTCGC	GTAACTTGGG	700
TAAGGTCATC	GATACCCTTA	CATGCGGCTT	CGCCGATCTC	ATGGGGTACA	750
TTCCGCTCGT	CGGCGCCCCC	CTAGGGGGCG	CTGCCAGGGC	CTTGGCACAC	800
GGTGTCCGGG	TTCTGGAGGA	CGGCGTGAAC	TATGCAACAG	GGAACTTGCC	850
CGGTTGCTCT	TTCTCTATCT	TCCTCTTGGC	TCTGCTGTCC	TGTTTGACCA	900
TCCCAGCTTC	CGCTTATGAA	GTGCGCAACG	TGTCCGGGAT	ATACCATGTC	950
ACGAACGACT	GCTCCAACTC	AAGCATTGTG	TATGAGGCAG	CGGACGTGAT	1000
CATGCATACT	CCCGGGTGCG	TGCCCTGTGT	TCAGGAGGGT	AACAGCTCCC	1050
GTTGCTGGGT	AGCGCTCACT	CCCACGCTCG	CGGCCAGGAA	TGCCAGCGTC	1100
CCCACTACGA	CAATACGACG	CCACGTCGAC	TTGCTCGTTG	GGACGGCTGC	1150
TTTCTGCTCC	GCTATGTACG	TGGGGGATCT	CTGCGGATCT	ATTTTCCTCG	1200
TCTCCCAGCT	GTTCACCTTC	TCGCCTCGCC	GGCATGAGAC	AGTGCAGGAC	1250
TGCAACTGCT	CAATCTATCC	CGGCCATGTA	TCAGGTCACC	GCATGGCTTG	1300
GGATATGATG	ATGAACTGGT	CACCTACAAC	AGCCCTAGTG	GTGTCGCAGT	1350
TGCTCCGGAT	CCCACAAGCT	GTCGTGGACA	TGGTGGCGGG	GGCCCACTGG	1400
GGAGTCCTGG	CGGGCCTTGC	CTACTATTCC	ATGGTAGGGA	ACTGGGCTAA	1450
GGTTCTGATT	GTGGCGCTAC	TCTTTGCCGG	CGTTGACGGG	GAGACCCACA	1500
CGACGGGGAG	GGTGGCCGGC	CACACCACCT	CCGGGTTCAC	GTCCCTTTTC	1550
TCATCTGGGG	CGTCTCAGAA	AATCCAGCTT	GTGAATACCA	ACGGCAGCTG	1600
GCACATCAAC	AGGACTGCCC	TAAATTGCAA	TGACTCCCTC	CAAACTGGGT	1650
TCTTTGCCGC	GCTGTTTTAC	GCACACAAGT	TCAACTCGTC	CGGGTGCCCG	1700
GAGCGCATGG	CCAGCTGCCG	CCCCATTGAC	TGGTTCGCCC	AGGGGTGGGG	1750
CCCCATCACC	TATACTAAGC	CTAACAGCTC	GGATCAGAGG	CCTTATTGCT	1800
GGCATTACGC	GCCTCGACCG	TGTGGTGTCG	TACCCGCGTC	GCAGGTGTGT	1850
GGTCCAGTGT	ATTGTTTCAC	CCCAAGCCCT	GTTGTGGTGG	GGACCACCGA	1900

FIG. 14A

HC-J4

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TCGTTCCGGT	GTCCCTACGT	ATAGCTGGGG	GGAGAATGAG	ACAGACGTGA	1950
TGCTCCTCAA	CAACACGCGT	CCGCCACAAG	GCAACTGGTT	CGGCTGTACA	2000
TGGATGAATA	GTACTGGGTT	CACTAAGACG	TGCGGAGGTC	CCCCGTGTAA	2050
CATCGGGGGG	GTCGGTAACC	GCACCTTGAT	CTGCCCCACG	GACTGCTTCC	2100
GGAAGCACCC	CGAGGCTACT	TACACAAAAT	GTGGCTCGGG	GCCCTGGTTG	2150
ACACCTAGGT	GCCTAGTAGA	CTACCCATAC	AGGCTTTGGC	ACTACCCCTG	2200
CACTCTCAAT	TTTTCCATCT	TTAAGGTTAG	GATGTATGTG	GGGGGCGTGG	2250
AGCACAGGCT	CAATGCCGCA	TGCAATTGGA	CTCGAGGAGA	GCGCTGTAAC	2300
TTGGAGGACA	GGGATAGGTC	AGAACTCAGC	CCGCTGCTGC	TGTCTACAAC	2350
AGAGTGGCAG	ATACTGCCCT	GTGCTTTCAC	CACCCTACCG	GCTTTATCCA	2400
CTGGTTTGAT	CCATCTCCAT	CAGAACATCG	TGGACGTGCA	ATACCTGTAC	2450
GGTGTAGGGT	CAGCGTTTGT	CTCCTTTGCA	ATCAAATGGG	AGTACATCCT	2500
GTTGCTTTTC	CTTCTCCTGG	CAGACGCGCG	CGTGTGTGCC	TGCTTGTGGA	2550
TGATGCTGCT	GATAGCCCAG	GCTGAGGCCG	CCTTAGAGAA	CTTGGTGGTC	2600
CTCAATGCGG	CGTCCGTGGC	CGGAGCGCAT	GGTATTCTCT	CCTTTCTTGT	2650
GTTCTTCTGC	GCCGCCTGGT	ACATTAAGGG	CAGGCTGGCT	CCTGGGGCGG	2700
CGTATGCTTT	TTATGGCGTA	TGGCCGCTGC	TCCTGCTCCT	ACTGGCGTTA	2750
CCACCACGAG	CTTACGCCTT	GGACCGGGAG	ATGGCTGCAT	CGTGCGGGGG	2800
TGCGGTTCTT	GTAGGTCTGG	TATTCTTGAC	CTTGTCACCA	TACTACAAAG	2850
TGTTTCTCAC	TAGGCTCATA	TGGTGGTTAC	AATACTTTAT	CACCAGAGCC	2900
GAGGCGCACA	TGCAAGTGTG	GGTCCCCCCC	CTCAACGTTC	GGGGAGGCCG	2950
CGATGCCATC	ATCCTCCTCA	CGTGTGCGGT	TCATCCAGAG	TTAATTTTTG	3000
ACATCACCAA	ACTCCTGCTC	GCCATACTCG	GCCCGCTCAT	GGTGCTCCAG	3050
GCTGGCATAA	CGAGAGTGCC	GTACTTCGTG	CGCGCTCAAG	GGCTCATTCG	3100
TGCATGCATG	TTAGTGCGAA	AAGTCGCCGG	GGGTCATTAT	GTCCAAATGG	3150
TCTTCATGAA	GCTGGGCGCG	CTGACAGGTA	CGTACGTTTA	TAACCATCTT	3200
ACCCCACTGC	GGGACTGGGC	CCACGCGGGC	CTACGAGACC	TTGCGGTGGC	3250
GGTAGAGCCC	GTCGTCTTCT	CCGCCATGGA	GACCAAGGTC	ATCACCTGGG	3300
GAGCAGACAC	CGCTGCGTGT	GGGGACATCA	TCTTGGGTCT	ACCCGTCTCC	3350
GCCCGAAGGG	GGAAGGAGAT	ATTTTTGGGA	CCGGCTGATA	GTCTCGAAGG	3400
GCAAGGGTGG	CGACTCCTTG	CGCCCATCAC	GGCCTACTCC	CAACAAACGC	3450
GGGGCGTACT	TGGTTGCATC	ATCACTAGCC	TCACAGGCCG	GGACAAGAAC	3500
CAGGTCGAAG	GGGAGGTTCA	AGTGGTTTCT	ACCGCAACAC	AATCTTTCCT	3550
GGCGACCTGC	ATCAACGGCG	TGTGCTGGAC	TGTCTACCAT	GGCGCTGGCT	3600
CGAAGACCCT	AGCCGGTCCA	AAAGGTCCAA	TCACCCAAAT	GTACACCAAT	3650
GTAGACCTGG	ACCTCGTCGG	CTGGCAGGCG	CCCCCCGGGG	CGCGCTCCAT	3700
GACACCATGC	AGCTGTGGCA	GCTCGGACCT	TTACTTGGTC	ACGAGACATG	3750
CTGATGTCAT	TCCGGTGCGC	CGGCGAGGCG	ACAGCAGGGG	AAGTCTACTC	3800

FIG. 14B

HC-J4

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
TCCCCCAGGC	CCGTCTCCTA	CCTGAAAGGC	TCCTCGGGTG	GTCCATTGCT	3850
TTGCCCTTCG	GGGCACGTCG	TGGGCGTCTT	CCGGGCTGCT	GTGTGCACCC	3900
GGGGGGTCGC	GAAGGCGGTG	GACTTCATAC	CCGTTGAGTC	TATGGAAACT	3950
ACCATGCGGT	CTCCGGTCTT	CACAGACAAC	TCAACCCCCC	CGGCTGTACC	4000
GCAGACATTC	CAAGTGGCAC	ATCTGCACGC	TCCTACTGGC	AGCGGCAAGA	4050
GCACCAAAGT	GCCGGCTGCG	TATGCAGCCC	AAGGGTACAA	GGTGCTCGTC	4100
CTGAACCCGT	CCGTTGCCGC	CACCTTAGGG	TTTGGGGCGT	ATATGTCCAA	4150
GGCACACGGT	ATCGACCCTA	ACATCAGAAC	TGGGGTAAGG	ACCATTACCA	4200
CGGGCGGCTC	CATTACGTAC	TCCACCTATG	GCAAGTTCCT	TGCCGACGGT	4250
GGCTGTTCTG	GGGGCGCCTA	TGACATCATA	ATATGTGATG	AGTGCCACTC	4300
AACTGACTCG	ACTACCATCT	TGGGCATCGG	CACAGTCCTG	GACCAAGCGG	4350
AGACGGCTGG	AGCGCGGCTC	GTCGTGCTCG	CCACCGCTAC	ACCTCCGGGA	4400
TCGGTTACCG	TGCCACACCC	CAATATCGAG	GAAATAGGCC	TGTCCAACAA	4450
TGGAGAGATC	CCCTTCTATG	GCAAAGCCAT	CCCCATTGAG	GCCATCAAGG	4500
GGGGGAGGCA	TCTCATTTTC	TGCCATTCCA	AGAAGAAATG	TGACGAGCTC	4550
GCCGCAAAGC	TGACAGGCCT	CGGACTGAAC	GCTGTAGCAT	ATTACCGGGG	4600
CCTTGATGTG	TCCGTCATAC	CGCCTATCGG	AGACGTCGTT	GTCGTGGCAA	4650
CAGACGCTCT	AATGACGGGT	TTCACCGGCG	ATTTTGACTC	AGTGATCGAC	4700
TGCAATACAT	GTGTCACCCA	GACAGTCGAC	TTCAGCTTGG	ATCCCACCTT	4750
CACCATTGAG	ACGACGACCG	TGCCCCAAGA	CGCGGTGTCG	CGCTCGCAAC	4800
GGCGAGGTAG	AACTGGCAGG	GGTAGGAGTG	GCATCTACAG	GTTTGTGACT	4850
CCAGGAGAAC	GGCCCTCGGG	CATGTTCGAT	TCTTCGGTCC	TGTGTGAGTG	4900
CTATGACGCG	GGCTGTGCTT	GGTATGAGCT	CACGCCCGCT	GAGACCTCGG	4950
TTAGGTTGCG	GGCTTACCTA	AATACACCAG	GGTTGCCCGT	CTGCCAGGAC	5000
CATCTGGAGT	TCTGGGAGAG	CGTCTTCACA	GGCCTCACCC	ACATAGATGC	5050
CCACTTCCTG	TCCCAGACTA	AACAGGCAGG	AGACAACTTT	CCTTACCTGG	5100
TGGCATATCA	AGCTACAGTG	TGCGCCAGGG	CTCAAGCTCC	ACCTCCATCG	5150
TGGGACCAAA	TGTGGAAGTG	TCTCATACGG	CTGAAACCTA	CACTGCACGG	5200
GCCAACACCC	CTGCTGTATA	GGCTAGGAGC	CGTCCAAAAT	GAGGTCATCC	5250
TCACACACCC	CATAACTAAA	TACATCATGG	CATGCATGTC	GGCTGACCTG	5300
GAGGTCGTCA	CTAGCACCTG	GGTGCTGGTA	GGCGGAGTCC	TTGCAGCTTT	5350
GGCCGCATAC	TGCCTGACGA	CAGGCAGTGT	GGTCATTGTG	GGCAGGATCA	5400
TCTTGTCCGG	GAAGCCAGCT	GTCGTTCCCG	ACAGGGAAGT	CCTCTACCAG	5450
GAGTTCGATG	AGATGGAAGA	GTGTGCCTCA	CAACTTCCTT	ACATCGAGCA	5500
GGGAATGCAG	CTCGCCGAGC	AATTCAAGCA	AAAGGCGCTC	GGGTTGTTGC	5550
AAACGGCCAC	CAAGCAAGCG	GAGGCTGCTG	CTCCCGTGGT	GGAGTCCAAG	5600
TGGCGAGCCC	TTGAGACCTT	CTGGGCGAAG	CACATGTGGA	ATTTCATCAG	5650
CGGAATACAG	TACCTAGCAG	GCTTATCCAC	TCTGCCTGGA	AACCCCGCGA	5700

FIG. 14C

HC-J4

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
TAGCATCATT	GATGGCATTT	ACAGCTTCTA	TCACTAGCCC	GCTCACCACC	5750
CAAAACACCC	TCCTGTTTAA	CATCTTGGGG	GGATGGGTGG	CTGCCCAACT	5800
CGCTCCTCCC	AGCGCTGCGT	CAGCTTTCGT	GGGCGCCGGC	ATCGCCGGAG	5850
CGGCTGTTGG	CAGCATAGGC	CTTGGGAAGG	TGCTCGTGGA	CATCTTGGCG	5900
GGCTATGGGG	CAGGGGTAGC	CGGCGCACTC	GTGGCCTTTA	AGGTCATGAG	5950
CGGCGAGGTG	CCCTCCACCG	AGGACCTGGT	CAACTTACTC	CCTGCCATCC	6000
TCTCTCCTGG	TGCCCTGGTC	GTCGGGGTCG	TGTGCGCAGC	AATACTGCGT	6050
CGGCACGTGG	GCCCGGGAGA	GGGGGCTGTG	CAGTGGATGA	ACCGGCTGAT	6100
AGCGTTCGCT	TCGCGGGGTA	ACCACGTCTC	CCCTACGCAC	TATGTGCCTG	6150
AGAGCGACGC	TGCAGCACGT	GTCACTCAGA	TCCTCTCTAG	CCTTACCATC	6200
ACTCAACTGC	TGAAGCGGCT	CCACCAGTGG	ATTAATGAGG	ACTGCTCTAC	6250
GCCATGCTCC	GGCTCGTGGC	TAAGGGATGT	TTGGGATTGG	ATATGCACGG	6300
TGTTGACTGA	CTTCAAGACC	TGGCTCCAGT	CCAAACTCCT	GCCGCGGTTA	6350
CCGGGAGTCC	CTTTCCTGTC	ATGCCAACGC	GGGTACAAGG	GAGTCTGGCG	6400
GGGGGACGGC	ATCATGCAAA	CCACCTGCCC	ATGCGGAGCA	CAGATCGCCG	6450
GACATGTCAA	AAACGGTTCC	ATGAGGATCG	TAGGGCCTAG	AACCTGCAGC	6500
AACACGTGGC	ACGGAACGTT	CCCCATCAAC	GCATACACCA	CGGGACCTTG	6550
CACACCCTCC	CCGGCGCCCA	ACTATTCCAG	GGCGCTATGG	CGGGTGGCTG	6600
CTGAGGAGTA	CGTGGAGGTT	ACGCGTGTGG	GGGATTTCCA	CTACGTGACG	6650
GGCATGACCA	CTGACAACGT	AAAGTGCCCA	TGCCAGGTTC	CGGCCCCCGA	6700
ATTCTTCACG	GAGGTGGATG	GAGTGCGGTT	GCACAGGTAC	GCTCCGGCGT	6750
GCAAACCTCT	TCTACGGGAG	GACGTCACGT	TCCAGGTCGG	GCTCAACCAA	6800
TACTTGGTCG	GGTCGCAGCT	CCCATGCGAG	CCCGAACCGG	ACGTAACAGT	6850
GCTTACTTCC	ATGCTCACCG	ATCCCTCCCA	CATTACAGCA	GAGACGGCTA	6900
AGCGTAGGCT	GGCTAGAGGG	TCTCCCCCCT	CTTTAGCCAG	CTCATCAGCT	6950
AGCCAGTTGT	CTGCGCCTTC	TTTGAAGGCG	ACATGCACTA	CCCACCATGA	7000
CTCCCCGGAC	GCTGACCTCA	TCGAGGCCAA	CCTCTTGTGG	CGGCAGGAGA	7050
TGGGCGGAAA	CATCACTCGC	GTGGAGTCAG	AGAATAAGGT	AGTAATTCTG	7100
GACTCTTTCG	AACCGCTTCA	CGCGGAGGGG	GATGAGAGGG	AGATATCCGT	7150
CGCGGCGGAG	ATCCTGCGAA	AATCCAGGAA	GTTCCCCTCA	GCGTTGCCCA	7200
TATGGGCACG	CCCGGACTAC	AATCCTCCAC	TGCTAGAGTC	CTGGAAGGAC	7250
CCGGACTACG	TCCCTCCGGT	GGTACACGGA	TGCCCATTGC	CACCTACCAA	7300
GGCTCCTCCA	ATACCACCTC	CACGGAGAAA	GAGGACGGTT	GTCCTGACAG	7350
AATCCAATGT	GTCTTCTGCC	TTGGCGGAGC	TCGCCACTAA	GACCTTCGGT	7400
AGCTCCGGAT	CGTCGGCCGT	TGATAGCGGC	ACGGCGACCG	CCCTTCCTGA	7450
CCTGGCCTCC	GACGACGGTG	ACAAAGGATC	CGACGTTGAG	TCGTACTCCT	7500
CCATGCCCCC	CCTTGAAGGG	GAGCCGGGGG	ACCCCGATCT	CAGCGACGGG	7550
TCTTGGTCTA	CCGTGAGTGA	GGAGGCTAGT	GAGGATGTCG	TCTGCTGCTC	7600

FIG. 14D

HC-J4

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
AATGTCCTAT	ACGTGGACAG	GCGCCCTGAT	CACGCCATGC	GCTGCGGAGG	7650
AAAGTAAGCT	GCCCATCAAC	CCGTTGAGCA	ACTCTTTGCT	GCGTCACCAC	7700
AACATGGTCT	ACGCCACAAC	ATCCCGCAGC	GCAAGCCTCC	GGCAGAAGAA	7750
GGTCACCTTT	GACAGATTGC	AAGTCCTGGA	TGATCATTAC	CGGGACGTAC	7800
TCAAGGAGAT	GAAGGCGAAG	GCGTCCACAG	TTAAGGCTAA	GCTTCTATCT	7850
ATAGAGGAGG	CCTGCAAGCT	GACGCCCCCA	CATTCGGCCA	AATCCAAATT	7900
TGGCTATGGG	GCAAAGGACG	TCCGGAACCT	ATCCAGCAGG	GCCGTTAACC	7950
ACATCCGCTC	CGTGTGGGAG	GACTTGCTGG	AAGACACTGA	AACACCAATT	8000
GACACCACCA	TCATGGCAAA	AAGTGAGGTT	TTCTGCGTCC	AACCAGAGAA	8050
GGGAGGCCGC	AAGCCAGCTC	GCCTTATCGT	ATTCCCAGAC	CTGGGAGTTC	8100
GTGTATGCGA	GAAGATGGCC	CTTTACGACG	TGGTCTCCAC	CCTTCCTCAG	8150
GCCGTGATGG	GCTCCTCATA	CGGATTTCAA	TACTCCCCCA	AGCAGCGGGT	8200
CGAGTTCCTG	GTGAATACCT	GGAAATCAAA	GAAATGCCCT	ATGGGCTTCT	8250
CATATGACAC	CCGCTGTTTT	GACTCAACGG	TCACTGAGAG	TGACATTCGT	8300
GTTGAGGAGT	CAATTTACCA	ATGTTGTGAC	TTGGCCCCCG	AGGCCAGACA	8350
GGCCATAAGG	TCGCTCACAG	AGCGGCTTTA	CATCGGGGGT	CCCCTGACTA	8400
ACTCAAAAGG	GCAGAACTGC	GGTTATCGCC	GGTGCCGCGC	AAGTGGCGTG	8450
CTGACGACTA	GCTGCGGTAA	TACCCTCACA	TGTTACTTGA	AGGCCACTGC	8500
AGCCTGTCGA	GCTGCAAAGC	TCCAGGACTG	CACGATGCTC	GTGAACGGAG	8550
ACGACCTTGT	CGTTATCTGT	GAAAGCGCGG	GAACCCAGGA	GGATGCGGCG	8600
GCCCTACGAG	CCTTCACGGA	GGCTATGACT	AGGTATTCCG	CCCCCCCCGG	8650
GGATCCGCCC	CAACCAGAAT	ACGACCTGGA	GCTGATAACA	TCATGTTCCT	8700
CCAATGTGTC	AGTCGCGCAC	GATGCATCTG	GCAAAAGGGT	ATACTACCTC	8750
ACCCGTGACC	CCACCACCCC	CCTTGCACGG	GCTGCGTGGG	AGACAGCTAG	8800
ACACACTCCA	ATCAACTCTT	GGCTAGGCAA	TATCATCATG	TATGCGCCCA	8850
CCCTATGGGC	AAGGATGATT	CTGATGACTC	ACTTTTTCTC	CATCCTTCTA	8900
GCTCAAGAGC	AACTTGAAAA	AGCCCTGGAT	TGTCAGATCT	ACGGGGCTTG	8950
CTACTCCATT	GAGCCACTTG	ACCTACCTCA	GATCATTGAA	CGACTCCATG	9000
GTCTTAGCGC	ATTTACACTC	CACAGTTACT	CTCCAGGTGA	GATCAATAGG	9050
GTGGCTTCAT	GCCTCAGGAA	ACTTGGGGTA	CCACCCTTGC	GAACCTGGAG	9100
ACATCGGGCC	AGAAGTGTCC	GCGCTAAGCT	ACTGTCCCAG	GGGGGGAGGG	9150
CCGCCACTTG	TGGCAGATAC	CTCTTTAACT	GGGCAGTAAG	GACCAAGCTT	9200
AAACTCACTC	CAATCCCGGC	CGCGTCCCAG	CTGGACTTGT	CTGGCTGGTT	9250
CGTCGCTGGT	TACAGCGGGG	GAGACATATA	TCACAGCCTG	TCTCGTGCCC	9300
GACCCCGCTG	GTTTCCGTTG	TGCCTACTCC	TACTTTCTGT	AGGGGTAGGC	9350
ATTTACCTGC	TCCCCAACCG	ATGAACGGGG	AGCTAACCAC	TCCAGGCCTT	9400
AAGCCATTTC	CTGTTTTTTT	TTTTTTTTTT	TTTTTTTTTT	TCTTTTTTTT	9450
TTTCTTTCCT	TTCCTTCTTT	TTTTCCTTTC	TTTTTCCCTT	CTTTAATGGT	9500

FIG. 14E

HC-J4

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
GGCTCCATCT	TAGCCCTAGT	CACGGCTAGC	TGTGAAAGGT	CCGTGAGCCG	9550
CATGACTGCA	GAGAGTGCTG	ATACTGGCCT	CTCTGCAGAT	CATGT	9595

FIG. 14F

HC-J4

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
MSTNPKPQRK	TKRNTNRRPQ	DVKFPGGGQI	VGGVYLLPRR	GPRLGVRATR	50
KASERSQPRG	RRQPIPKARR	PEGRAWAQPG	YPWPLYGNEG	LGWAGWLLSP	100
RGSRPSWGPT	DPRRRSRNLG	KVIDTLTCGF	ADLMGYIPLV	GAPLGGAARA	150
LAHGVRVLED	GVNYATGNLP	GCSFSIFLLA	LLSCLTIPAS	AYEVRNVSGI	200
YHVTNDCSNS	SIVYEAADVI	MHTPGCVPCV	QEGNSSRCWV	ALTPTLAARN	250
ASVPTTTIRR	HVDLLVGTAA	FCSAMYVGDL	CGSIFLVSQL	FTFSPRRHET	300
VQDCNCSIYP	GHVSGHRMAW	DMMMNWSPTT	ALVVSQLLRI	PQAVVDMVAG	350
AHWGVLAGLA	YYSMVGNWAK	VLIVALLFAG	VDGETHTTGR	VAGHTTSGFT	400
SLFSSGASQK	IQLVNTNGSW	HINRTALNCN	DSLQTGFFAA	LFYAHKFNSS	450
GCPERMASCR	PIDWFAQGWG	PITYTKPNSS	DQRPYCWHYA	PRPCGVVPAS	500
QVCGPVYCFT	PSPVVVGTTD	RSGVPTYSWG	ENETDVMLLN	NTRPPQGNWF	550
GCTWMNSTGF	TKTCGGPPCN	IGGVGNRTLI	CPTDCFRKHP	EATYTKCGSG	600
PWLTPRCLVD	YPYRLWHYPC	TLNFSIFKVR	MYVGGVEHRL	NAACNWTRGE	650
RCNLEDRDRS	ELSPLLLSTT	EWQILPCAFT	TLPALSTGLI	HLHQNIVDVQ	700
YLYGVGSAFV	SFAIKWEYIL	LLFLLLADAR	VCACLWMMLL	IAQAEAALEN	750
LVVLNAASVA	GAHGILSFLV	FFCAAWYIKG	RLAPGAAYAF	YGVWPLLLLL	800
LALPPRAYAL	DREMAASCGG	AVLVGLVFLT	LSPYYKVFLT	RLIWWLQYFI	850
TRAEAHMQVW	VPPLNVRGGR	DAIILLTCAV	HPELIFDITK	LLLAILGPLM	900
VLQAGITRVP	YFVRAQGLIR	ACMLVRKVAG	GHYVQMVFMK	LGALTGTYVY	950
NHLTPLRDWA	HAGLRDLAVA	VEPVVFSAME	TKVITWGADT	AACGDIILGL	1000
PVSARRGKEI	FLGPADSLEG	QGWRLLAPIT	AYSQQTRGVL	GCIITSLTGR	1050
DKNQVEGEVQ	VVSTATQSFL	ATCINGVCWT	VYHGAGSKTL	AGPKGPITQM	1100
YTNVDLDLVG	WQAPPGARSM	TPCSCGSSDL	YLVTRHADVI	PVRRRGDSRG	1150
SLLSPRPVSY	LKGSSGGPLL	CPSGHVVGVF	RAAVCTRGVA	KAVDFIPVES	1200
METTMRSPVF	TDNSTPPAVP	QTFQVAHLHA	PTGSGKSTKV	PAAYAAQGYK	1250
VLVLNPSVAA	TLGFGAYMSK	AHGIDPNIRT	GVRTITTGGS	ITYSTYGKFL	1300
ADGGCSGGAY	DIIICDECHS	TDSTTILGIG	TVLDQAETAG	ARLVVLATAT	1350
PPGSVTVPHP	NIEEIGLSNN	GEIPFYGKAI	PIEAIKGGRH	LIFCHSKKKC	1400
DELAAKLTGL	GLNAVAYYRG	LDVSVIPPIG	DVVVVATDAL	MTGFTGDFDS	1450
VIDCNTCVTQ	TVDFSLDPTF	TIETTTVPQD	AVSRSQRRGR	TGRGRSGIYR	1500
FVTPGERPSG	MFDSSVLCEC	YDAGCAWYEL	TPAETSVRLR	AYLNTPGLPV	1550
CQDHLEFWES	VFTGLTHIDA	HFLSQTKQAG	DNFPYLVAYQ	ATVCARAQAP	1600
PPSWDQMWKC	LIRLKPTLHG	PTPLLYRLGA	VQNEVILTHP	ITKYIMACMS	1650
ADLEVVTSTW	VLVGGVLAAL	AAYCLTTGSV	VIVGRIILSG	KPAVVPDREV	1700
LYQEFDEMEE	CASQLPYIEQ	GMQLAEQFKQ	KALGLLQTAT	KQAEAAAPVV	1750
ESKWRALETF	WAKHMWNFIS	GIQYLAGLST	LPGNPAIASL	MAFTASITSP	1800
LTTQNTLLFN	ILGGWVAAQL	APPSAASAFV	GAGIAGAAVG	SIGLGKVLVD	1850
ILAGYGAGVA	GALVAFKVMS	GEVPSTEDLV	NLLPAILSPG	ALVVGVVCAA	1900

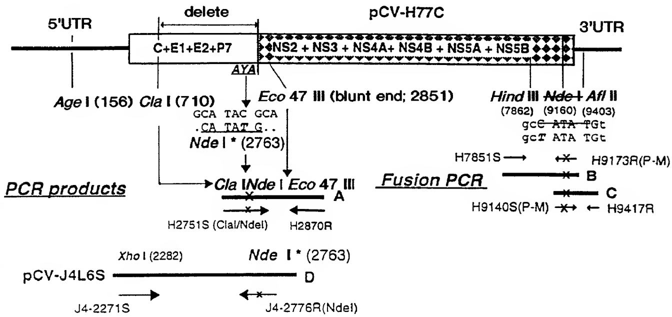
FIG. 14G

HC-J4

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
ILRRHVGPGE	GAVQWMNRLI	AFASRGNHVS	PTHYVPESDA	AARVTQILSS	1950
LTITQLLKRL	HQWINEDCST	PCSGSWLRDV	WDWICTVLTD	FKTWLQSKLL	2000
PRLPGVPFLS	CQRGYKGVWR	GDGIMQTTCP	CGAQIAGHVK	NGSMRIVGPR	2050
TCSNTWHGTF	PINAYTTGPC	TPSPAPNYSR	ALWRVAAEEY	VEVTRVGDFH	2100
YVTGMTTDNV	KCPCQVPAPE	FFTEVDGVRL	HRYAPACKPL	LREDVTFQVG	2150
LNQYLVGSQL	PCEPEPDVTV	LTSMLTDPSH	ITAETAKRRL	ARGSPPSLAS	2200
SSASQLSAPS	LKATCTTHHD	SPDADLIEAN	LLWRQEMGGN	ITRVESENKV	2250
VILDSFEPLH	AEGDEREISV	AAEILRKSRK	FPSALPIWAR	PDYNPPLLES	2300
WKDPDYVPPV	VHGCPLPPTK	APPIPPPRRK	RTVVLTESNV	SSALAELATK	2350
TFGSSGSSAV	DSGTATALPD	LASDDGDKGS	DVESYSSMPP	LEGEPGDPDL	2400
SDGSWSTVSE	EASEDVVCCS	MSYTWTGALI	TPCAAEESKL	PINPLSNSLL	2450
RHHNMVYATT	SRSASLRQKK	VTFDRLQVLD	DHYRDVLKEM	KAKASTVKAK	2500
LLSIEEACKL	TPPHSAKSKF	GYGAKDVRNL	SSRAVNHIRS	VWEDLLEDTE	2550
TPIDTTIMAK	SEVFCVQPEK	GGRKPARLIV	FPDLGVRVCE	KMALYDVVST	2600
LPQAVMGSSY	GFQYSPKQRV	EFLVNTWKSK	KCPMGFSYDT	RCFDSTVTES	2650
DIRVEESIYQ	CCDLAPEARQ	AIRSLTERLY	IGGPLTNSKG	QNCGYRRCRA	2700
SGVLTTSCGN	TLTCYLKATA	ACRAAKLQDC	TMLVNGDDLV	VICESAGTQE	2750
DAAALRAFTE	AMTRYSAPPG	DPPQPEYDLE	LITSCSSNVS	VAHDASGKRV	2800
YYLTRDPTTP	LARAAWETAR	HTPINSWLGN	IIMYAPTLWA	RMILMTHFFS	2850
ILLAQEQLEK	ALDCQIYGAC	YSIEPLDLPQ	IIERLHGLSA	FTLHSYSPGE	2900
INRVASCLRK	LGVPPLRTWR	HRARSVRAKL	LSQGGRAATC	GRYLFNWAVR	2950
TKLKLTPIPA	ASQLDLSGWF	VAGYSGGDIY	HSLSRARPRW	FPLCLLLLSV	3000
GVGIYLLPNR					3010

FIG. 14H

#2. Strategy for constructing chimeric clone of HCV (pH77CV-J4) which contains the nonstructural region of strain H77 and the structural region of strain HC-J4



1. Fragment A, B, C and D ; PCR amplification from pCV-H77C or pCV-J4L6S
   - Fragment A ; additional *Cla* I site, artificial *Nde* I site induced by a single mutation (C→T at nt 2765 of H77C) and authentic *Eco*47 III site
   - Fragment B and C ; eliminated Nde I site by a single mutation within the primers (C→T at nt 9158 of H77C) , and fusion PCR with both fragments
   - Fragment D ; artificial *Nde* I site induced by 2 point mutations within the primer (T→A at nt 2762 and C→T at nt 2765 of J4L6S)
2. TA cloning of PCR products
3. Sequence analysis
4. Cloning of Fragment A (*Cla* I-*Eco* 47III) and Fragment B/C (*Hind* III-*Afl* II) with correct sequence into pCV-H77C
5. Complete sequence analysis of new cassette vector [pH77CV], into which the structural regions of different genotypes can be inserted.
6. Cloning of Fragment-*Age* I/*Xho* I (cut out from pCV-J4L6S) and Fragment D (*Xho* I-*Nde* I) with correct sequence into the new cassette vector ; 3 piece ligation
7. Complete sequence analysis of 1a+1b chimera [pH77CV-J4]
8. *In vitro* transcription (within 24 hours of inoculation)
9. Percutaneous intrahepatic transfection into chimpanzee

FIG. 15

pH77CV-J4 Sequence

```
GCCAGCCCCC TGATGGGGGC GACACTCCAC CATGAATCAC TCCCCTGTGA     50
GGAACTACTG TCTTCACGCA GAAAGCGTCT AGCCATGGCG TTAGTATGAG    100
TGTCGTGCAG CCTCCAGGAC CCCCCCTCCC GGGAGAGCCA TAGTGGTCTG    150
CGGAACCGGT GAGTACACCG GAATTGCCAG GACGACCGGG TCCTTTCTTG    200
GATCAACCCG CTCAATGCCT GGAGATTTGG GCGTGCCCCC GCGAGACTGC    250
TAGCCGAGTA GTGTTGGGTC GCGAAAGGCC TTGTGGTACT GCCTGATAGG    300
GTGCTTGCGA GTGCCCCGGG AGGTCTCGTA GACCGTGCAC CATGAGCACG    350
AATCCTAAAC CTCAAAGAAA AACCAAACGT AACACCAACC GCCGCCCACA    400
GGACGTCAAG TTCCCGGGCG GTGGTCAGAT CGTTGGTGGA GTTTACCTGT    450
TGCCGCGCAG GGGCCCCAGG TTGGGTGTGC GCGCGACTAG GAAGGCTTCC    500
GAGCGGTCGC AACCTCGTGG AAGGCGACAA CCTATCCCAA AGGCTCGCCG    550
ACCCGAGGGC AGGGCCTGGG CTCAGCCCGG GTACCCTTGG CCCCTCTATG    600
GCAATGAGGG CCTGGGGTGG GCAGGATGGC TCCTGTCACC CCGCGGCTCC    650
CGGCCTAGTT GGGGCCCCAC GGACCCCCGG CGTAGGTCGC GTAACTTGGG    700
TAAGGTCATC GATACCCTTA CATGCGGCTT CGCCGATCTC ATGGGGTACA    750
TTCCGCTCGT CGGCGCCCCC CTAGGGGGCG CTGCCAGGGC CTTGGCACAC    800
GGTGTCCGGG TTCTGGAGGA CGGCGTGAAC TATGCAACAG GGAACTTGCC    850
CGGTTGCTCT TTCTCTATCT TCCTCTTGGC TCTGCTGTCC TGTTTGACCA    900
TCCCAGCTTC CGCTTATGAA GTGCGCAACG TGTCCGGGAT ATACCATGTC    950
ACGAACGACT GCTCCAACTC AAGCATTGTG TATGAGGCAG CGGACGTGAT   1000
CATGCATACT CCCGGGTGCG TGCCCTGTGT TCAGGAGGGT AACAGCTCCC   1050
GTTGCTGGGT AGCGCTCACT CCCACGCTCG CGGCCAGGAA TGCCAGCGTC   1100
CCCACTACGA CAATACGACG CCACGTCGAC TTGCTCGTTG GGACGGCTGC   1150
TTTCTGCTCC GCTATGTACG TGGGGGATCT CTGCGGATCT ATTTTCCTCG   1200
TCTCCCAGCT GTTCACCTTC TCGCCTCGCC GGCATGAGAC AGTGCAGGAC   1250
TGCAACTGCT CAATCTATCC CGGCCATGTA TCAGGTCACC GCATGGCTTG   1300
GGATATGATG ATGAACTGGT CACCTACAAC AGCCCTAGTG GTGTCGCAGT   1350
TGCTCCGGAT CCCACAAGCT GTCGTGGACA TGGTGGCGGG GGCCCACTGG   1400
GGAGTCCTGG CGGGCCTTGC CTACTATTCC ATGGTAGGGA ACTGGGCTAA   1450
GGTTCTGATT GTGGCGCTAC TCTTTGCCGG CGTTGACGGG GAGACCCACA   1500
CGACGGGGAG GGTGGCCGGC CACACCACCT CCGGGTTCAC GTCCCTTTTC   1550
TCATCTGGGG CGTCTCAGAA AATCCAGCTT GTGAATACCA ACGGCAGCTG   1600
GCACATCAAC AGGACTGCCC TAAATTGCAA TGACTCCCTC CAAACTGGGT   1650
TCTTTGCCGC GCTGTTTTAC GCACACAAGT TCAACTCGTC CGGGTGCCCG   1700
GAGCGCATGG CCAGCTGCCG CCCCATTGAC TGGTTCGCCC AGGGGTGGGG   1750
CCCCATCACC TATACTAAGC CTAACAGCTC GGATCAGAGG CCTTATTGCT   1800
```

FIG. 16A

pH77CV-J4 Sequence

```
GGCATTACGC GCCTCGACCG TGTGGTGTCG TACCCGCGTC GCAGGTGTGT   1850
GGTCCAGTGT ATTGTTTCAC CCCAAGCCCT GTTGTGGTGG GGACCACCGA   1900
TCGTTCCGGT GTCCCTACGT ATAGCTGGGG GGAGAATGAG ACAGACGTGA   1950
TGCTCCTCAA CAACACGCGT CCGCCACAAG GCAACTGGTT CGGCTGTACA   2000
TGGATGAATA GTACTGGGTT CACTAAGACG TGCGGAGGTC CCCCGTGTAA   2050
CATCGGGGGG GTCGGTAACC GCACCTTGAT CTGCCCCACG GACTGCTTCC   2100
GGAAGCACCC CGAGGCTACT TACACAAAAT GTGGCTCGGG GCCCTGGTTG   2150
ACACCTAGGT GCCTAGTAGA CTACCCATAC AGGCTTTGGC ACTACCCCTG   2200
CACTCTCAAT TTTTCCATCT TTAAGGTTAG GATGTATGTG GGGGGCGTGG   2250
AGCACAGGCT CAATGCCGCA TGCAATTGGA CTCGAGGAGA GCGCTGTAAC   2300
TTGGAGGACA GGGATAGGTC AGAACTCAGC CCGCTGCTGC TGTCTACAAC   2350
AGAGTGGCAG ATACTGCCCT GTGCTTTCAC CACCCTACCG GCTTTATCCA   2400
CTGGTTTGAT CCATCTCCAT CAGAACATCG TGGACGTGCA ATACCTGTAC   2450
GGTGTAGGGT CAGCGTTTGT CTCCTTTGCA ATCAAATGGG AGTACATCCT   2500
GTTGCTTTTC CTTCTCCTGG CAGACGCGCG CGTGTGTGCC TGCTTGTGGA   2550
TGATGCTGCT GATAGCCCAG GCTGAGGCCG CCTTAGAGAA CTTGGTGGTC   2600
CTCAATGCGG CGTCCGTGGC CGGAGCGCAT GGTATTCTCT CCTTTCTTGT   2650
GTTCTTCTGC GCCGCCTGGT ACATTAAGGG CAGGCTGGCT CCTGGGGCGG   2700
CGTATGCTTT TTATGGCGTA TGGCCGCTGC TCCTGCTCCT ACTGGCGTTA   2750
CCACCACGAG CATATGCACT GGACACGGAG GTGGCCGCGT CGTGTGGCGG   2800
CGTTGTTCTT GTCGGGTTAA TGGCGCTGAC TCTGTCGCCA TATTACAAGC   2850
GCTATATCAG CTGGTGCATG TGGTGGCTTC AGTATTTTCT GACCAGAGTA   2900
GAAGCGCAAC TGCACGTGTG GGTTCCCCCC CTCAACGTCC GGGGGGGCCG   2950
CGATGCCGTC ATCTTACTCA TGTGTGTAGT ACACCCGACC CTGGTATTTG   3000
ACATCACCAA ACTACTCCTG GCCATCTTCG GACCCCTTTG GATTCTTCAA   3050
GCCAGTTTGC TTAAAGTCCC CTACTTCGTG CGCGTTCAAG GCCTTCTCCG   3100
GATCTGCGCG CTAGCGCGGA AGATAGCCGG AGGTCATTAC GTGCAAATGG   3150
CCATCATCAA GTTAGGGGCG CTTACTGGCA CCTATGTGTA TAACCATCTC   3200
ACCCCTCTTC GAGACTGGGC GCACAACGGC CTGCGAGATC TGGCCGTGGC   3250
TGTGGAACCA GTCGTCTTCT CCCGAATGGA GACCAAGCTC ATCACGTGGG   3300
GGGCAGATAC CGCCGCGTGC GGTGACATCA TCAACGGCTT GCCCGTCTCT   3350
GCCCGTAGGG GCCAGGAGAT ACTGCTTGGG CCAGCCGACG GAATGGTCTC   3400
CAAGGGGTGG AGGTTGCTGG CGCCCATCAC GGCGTACGCC CAGCAGACGA   3450
GAGGCCTCCT AGGGTGTATA ATCACCAGCC TGACTGGCCG GGACAAAAAC   3500
CAAGTGGAGG GTGAGGTCCA GATCGTGTCA ACTGCTACCC AAACCTTCCT   3550
GGCAACGTGC ATCAATGGGG TATGCTGGAC TGTCTACCAC GGGGCCGGAA   3600
```

FIG. 16B

pH77CV-J4 Sequence

CGAGGACCAT CGCATCACCC AAGGGTCCTG TCATCCAGAT GTATACCAAT 3650
GTGGACCAAG ACCTTGTGGG CTGGCCCGCT CCTCAAGGTT CCCGCTCATT 3700
GACACCCTGT ACCTGCGGCT CCTCGGACCT TTACCTGGTC ACGAGGCACG 3750
CCGATGTCAT TCCCGTGCGC CGGCGAGGTG ATAGCAGGGG TAGCCTGCTT 3800

TCGCCCCGGC CCATTTCCTA CTTGAAAGGC TCCTCGGGGG GTCCGCTGTT 3850
GTGCCCCGCG GGACACGCCG TGGGCCTATT CAGGGCCGCG GTGTGCACCC 3900
GTGGAGTGGC TAAAGCGGTG GACTTTATCC CTGTGGAGAA CCTAGGGACA 3950
ACCATGAGAT CCCCGGTGTT CACGGACAAC TCCTCTCCAC CAGCAGTGCC 4000
CCAGAGCTTC CAGGTGGCCC ACCTGCATGC TCCCACCGGC AGCGGTAAGA 4050
GCACCAAGGT CCCGGCTGCG TACGCAGCCC AGGGCTACAA GGTGTTGGTG 4100
CTCAACCCCT CTGTTGCTGC AACGCTGGGC TTTGGTGCTT ACATGTCCAA 4150
GGCCCATGGG GTTGATCCTA ATATCAGGAC CGGGGTGAGA ACAATTACCA 4200
CTGGCAGCCC CATCACGTAC TCCACCTACG GCAAGTTCCT TGCCGACGGC 4250
GGGTGCTCAG GAGGTGCTTA TGACATAATA ATTTGTGACG AGTGCCACTC 4300
CACGGATGCC ACATCCATCT TGGGCATCGG CACTGTCCTT GACCAAGCAG 4350
AGACTGCGGG GGCGAGACTG GTTGTGCTCG CCACTGCTAC CCCTCCGGGC 4400
TCCGTCACTG TGTCCCATCC TAACATCGAG GAGGTTGCTC TGTCCACCAC 4450
CGGAGAGATC CCCTTTTACG GCAAGGCTAT CCCCCTCGAG GTGATCAAGG 4500
GGGGAAGACA TCTCATCTTC TGCCACTCAA AGAAGAAGTG CGACGAGCTC 4550
GCCGCGAAGC TGGTCGCATT GGGCATCAAT GCCGTGGCCT ACTACCGCGG 4600
TCTTGACGTG TCTGTCATCC CGACCAGCGG CGATGTTGTC GTCGTGTCGA 4650
CCGATGCTCT CATGACTGGC TTTACCGGCG ACTTCGACTC TGTGATAGAC 4700
TGCAACACGT GTGTCACTCA GACAGTCGAT TTCAGCCTTG ACCCTACCTT 4750
TACCATTGAG ACAACCACGC TCCCCCAGGA TGCTGTCTCC AGGACTCAAC 4800
GCCGGGGCAG GACTGGCAGG GGGAAGCCAG GCATCTATAG ATTTGTGGCA 4850
CCGGGGGAGC GCCCCTCCGG CATGTTCGAC TCGTCCGTCC TCTGTGAGTG 4900
CTATGACGCG GGCTGTGCTT GGTATGAGCT CACGCCCGCC GAGACTACAG 4950
TTAGGCTACG AGCGTACATG AACACCCCGG GGCTTCCCGT GTGCCAGGAC 5000
CATCTTGAAT TTTGGGAGGG CGTCTTTACG GGCCTCACTC ATATAGATGC 5050
CCACTTTTTA TCCCAGACAA AGCAGAGTGG GGAGAACTTT CCTTACCTGG 5100
TAGCGTACCA AGCCACCGTG TGCGCTAGGG CTCAAGCCCC TCCCCCATCG 5150
TGGGACCAGA TGTGGAAGTG TTTGATCCGC CTTAAACCCA CCCTCCATGG 5200
GCCAACACCC CTGCTATACA GACTGGGCGC TGTTCAGAAT GAAGTCACCC 5250
TGACGCACCC AATCACCAAA TACATCATGA CATGCATGTC GGCCGACCTG 5300
GAGGTCGTCA CGAGCACCTG GGTGCTCGTT GGCGGCGTCC TGGCTGCTCT 5350
GGCCGCGTAT TGCCTGTCAA CAGGCTGCGT GGTCATAGTG GGCAGGATCG 5400

FIG. 16C

pH77CV-J4 Sequence

TCTTGTCCGG GAAGCCGGCA ATTATACCTG ACAGGGAGGT TCTCTACCAG 5450
GAGTTCGATG AGATGGAAGA GTGCTCTCAG CACTTACCGT ACATCGAGCA 5500
AGGGATGATG CTCGCTGAGC AGTTCAAGCA GAAGGCCCTC GGCCTCCTGC 5550
AGACCGCGTC CCGCCATGCA GAGGTTATCA CCCCTGCTGT CCAGACCAAC 5600
TGGCAGAAAC TCGAGGTCTT TTGGGCGAAG CACATGTGGA ATTTCATCAG 5650
TGGGATACAA TACTTGGCGG GCCTGTCAAC GCTGCCTGGT AACCCCGCCA 5700
TTGCTTCATT GATGGCTTTT ACAGCTGCCG TCACCAGCCC ACTAACCACT 5750
GGCCAAACCC TCCTCTTCAA CATATTGGGG GGGTGGGTGG CTGCCCAGCT 5800
CGCCGCCCCC GGTGCCGCTA CTGCCTTTGT GGGTGCTGGC CTAGCTGGCG 5850
CCGCCATCGG CAGCGTTGGA CTGGGGAAGG TCCTCGTGGA CATTCTTGCA 5900
GGGTATGGCG CGGGCGTGGC GGGAGCTCTT GTAGCATTCA AGATCATGAG 5950
CGGTGAGGTC CCCTCCACGG AGGACCTGGT CAATCTGCTG CCCGCCATCC 6000
TCTCGCCTGG AGCCCTTGTA GTCGGTGTGG TCTGCGCAGC AATACTGCGC 6050
CGGCACGTTG GCCCGGGCGA GGGGGCAGTG CAATGGATGA ACCGGCTAAT 6100
AGCCTTCGCC TCCCGGGGGA ACCATGTTTC CCCCACGCAC TACGTGCCGG 6150
AGAGCGATGC AGCCGCCCGC GTCACTGCCA TACTCAGCAG CCTCACTGTA 6200
ACCCAGCTCC TGAGGCGACT GCATCAGTGG ATAAGCTCGG AGTGTACCAC 6250
TCCATGCTCC GGTTCCTGGC TAAGGACAT CTGGGACTGG ATATGCGAGG 6300
TGCTGAGCGA CTTTAAGACC TGGCTGAAAG CCAAGCTCAT GCCACAACTG 6350
CCTGGGATTC CCTTTGTGTC CTGCCAGCGC GGGTATAGGG GGGTCTGGCG 6400
AGGAGACGGC ATTATGCACA CTCGCTGCCA CTGTGGAGCT GAGATCACTG 6450
GACATGTCAA AAACGGGACG ATGAGGATCG TCGGTCCTAG GACCTGCAGG 6500
AACATGTGGA GTGGGACGTT CCCCATTAAC GCCTACACCA CGGGCCCCTG 6550
TACTCCCCTT CCTGCGCCGA ACTATAAGTT CGCGCTGTGG AGGGTGTCTG 6600
CAGAGGAATA CGTGGAGATA AGGCGGGTGG GGGACTTCCA CTACGTATCG 6650
GGTATGACTA CTGACAATCT TAAATGCCCG TGCCAGATCC CATCGCCCGA 6700
ATTTTTCACA GAATTGGACG GGGTGCGCCT ACACAGGTTT GCGCCCCCTT 6750
GCAAGCCCTT GCTGCGGGAG GAGGTATCAT TCAGAGTAGG ACTCCACGAG 6800
TACCCGGTGG GGTCGCAATT ACCTTGCGAG CCCGAACCGG ACGTAGCCGT 6850
GTTGACGTCC ATGCTCACTG ATCCCTCCCA TATAACAGCA GAGGCGGCCG 6900
GGAGAAGGTT GGCGAGAGGG TCACCCCCTT CTATGGCCAG CTCCTCGGCT 6950
AGCCAGCTGT CCGCTCCATC TCTCAAGGCA ACTTGCACCG CCAACCATGA 7000
CTCCCCTGAC GCCGAGCTCA TAGAGGCTAA CCTCCTGTGG AGGCAGGAGA 7050
TGGGCGGCAA CATCACCAGG GTTGAGTCAG AGAACAAAGT GGTGATTCTG 7100
GACTCCTTCG ATCCGCTTGT GGCAGAGGAG GATGAGCGGG AGGTCTCCGT 7150
ACCTGCAGAA ATTCTGCGGA AGTCTCGGAG ATTCGCCCGG GCCCTGCCCG 7200

FIG. 16D

pH77CV-J4 Sequence

TCTGGGCGCG	GCCGGACTAC	AACCCCCCGC	TAGTAGAGAC	GTGGAAAAAG	7250
CCTGACTACG	AACCACCTGT	GGTCCATGGC	TGCCCGCTAC	CACCTCCACG	7300
GTCCCCTCCT	GTGCCTCCGC	CTCGGAAAAA	GCGTACGGTG	GTCCTCACCG	7350
AATCAACCCT	ATCTACTGCC	TTGGCCGAGC	TTGCCACCAA	AAGTTTTGGC	7400
AGCTCCTCAA	CTTCCGGCAT	TACGGGCGAC	AATACGACAA	CATCCTCTGA	7450
GCCCGCCCCT	TCTGGCTGCC	CCCCCGACTC	CGACGTTGAG	TCCTATTCTT	7500
CCATGCCCCC	CCTGGAGGGG	GAGCCTGGGG	ATCCGGATCT	CAGCGACGGG	7550
TCATGGTCGA	CGGTCAGTAG	TGGGGCCGAC	ACGGAAGATG	TCGTGTGCTG	7600
CTCAATGTCT	TATTCCTGGA	CAGGCGCACT	CGTCACCCCG	TGCGCTGCGG	7650
AAGAACAAAA	ACTGCCCATC	AACGCACTGA	GCAACTCGTT	GCTACGCCAT	7700
CACAATCTGG	TGTATTCCAC	CACTTCACGC	AGTGCTTGCC	AAAGGCAGAA	7750
GAAAGTCACA	TTTGACAGAC	TGCAAGTTCT	GGACAGCCAT	TACCAGGACG	7800
TGCTCAAGGA	GGTCAAAGCA	GCGGCGTCAA	AAGTGAAGGC	TAACTTGCTA	7850
TCCGTAGAGG	AAGCTTGCAG	CCTGACGCCC	CCACATTCAG	CCAAATCCAA	7900
GTTTGGCTAT	GGGGCAAAAG	ACGTCCGTTG	CCATGCCAGA	AAGGCCGTAG	7950
CCCACATCAA	CTCCGTGTGG	AAAGACCTTC	TGGAAGACAG	TGTAACACCA	8000
ATAGACACTA	CCATCATGGC	CAAGAACGAG	GTTTTCTGCG	TTCAGCCTGA	8050
GAAGGGGGGT	CGTAAGCCAG	CTCGTCTCAT	CGTGTTCCCC	GACCTGGGCG	8100
TGCGCGTGTG	CGAGAAGATG	GCCCTGTACG	ACGTGGTTAG	CAAGCTCCCC	8150
CTGGCCGTGA	TGGGAAGCTC	CTACGGATTC	CAATACTCAC	CAGGACAGCG	8200
GGTTGAATTC	CTCGTGCAAG	CGTGGAAGTC	CAAGAAGACC	CCGATGGGGT	8250
TCTCGTATGA	TACCCGCTGT	TTTGACTCCA	CAGTCACTGA	GAGCGACATC	8300
CGTACGGAGG	AGGCAATTTA	CCAATGTTGT	GACCTGGACC	CCCAAGCCCG	8350
CGTGGCCATC	AAGTCCCTCA	CTGAGAGGCT	TTATGTTGGG	GGCCCTCTTA	8400
CCAATTCAAG	GGGGGAAAAC	TGCGGCTACC	GCAGGTGCCG	CGCGAGCGGC	8450
GTACTGACAA	CTAGCTGTGG	TAACACCCTC	ACTTGCTACA	TCAAGGCCCG	8500
GGCAGCCTGT	CGAGCCGCAG	GGCTCCAGGA	CTGCACCATG	CTCGTGTGTG	8550
GCGACGACTT	AGTCGTTATC	TGTGAAAGTG	CGGGGGTCCA	GGAGGACGCG	8600
GCGAGCCTGA	GAGCCTTCAC	GGAGGCTATG	ACCAGGTACT	CCGCCCCCCC	8650
CGGGGACCCC	CCACAACCAG	AATACGACTT	GGAGCTTATA	ACATCATGCT	8700
CCTCCAACGT	GTCAGTCGCC	CACGACGGCG	CTGGAAAGAG	GGTCTACTAC	8750
CTTACCCGTG	ACCCTACAAC	CCCCCTCGCG	AGAGCCGCGT	GGGAGACAGC	8800
AAGACACACT	CCAGTCAATT	CCTGGCTAGG	CAACATAATC	ATGTTTGCCC	8850
CCACACTGTG	GGCGAGGATG	ATACTGATGA	CCCATTTCTT	TAGCGTCCTC	8900
ATAGCCAGGG	ATCAGCTTGA	ACAGGCTCTT	AACTGTGAGA	TCTACGGAGC	8950
CTGCTACTCC	ATAGAACCAC	TGGATCTACC	TCCAATCATT	CAAAGACTCC	9000

FIG. 16E

pH77CV-J4 Sequence

```
ATGGCCTCAG CGCATTTTCA CTCCACAGTT ACTCTCCAGG TGAAATCAAT    9050
AGGGTGGCCG CATGCCTCAG AAAACTTGGG GTCCCGCCCT TGCGAGCTTG    9100
GAGACACCGG GCCCGGAGCG TCCGCGCTAG GCTTCTGTCC AGAGGAGGCA    9150
GGGCTGCTAT ATGTGGCAAG TACCTCTTCA ACTGGGCAGT AAGAACAAAG    9200
CTCAAACTCA CTCCAATAGC GGCCGCTGGC CGGCTGGACT TGTCCGGTTG    9250
GTTCACGGCT GGCTACAGCG GGGGAGACAT TTATCACAGC GTGTCTCATG    9300
CCCGGCCCCG CTGGTTCTGG TTTTGCCTAC TCCTGCTCGC TGCAGGGGTA    9350
GGCATCTACC TCCTCCCCAA CCGATGAAGG TTGGGGTAAA CACTCCGGCC    9400
TCTTAAGCCA TTTCCTGTTT TTTTTTTTTT TTTTTTTTTT TTTTTCTTTT    9450
TTTTTTTCTT TCCTTTCCTT CTTTTTTTCC TTTCTTTTTC CCTTCTTTAA    9500
TGGTGGCTCC ATCTTAGCCC TAGTCACGGC TAGCTGTGAA AGGTCCGTGA    9550
GCCGCATGAC TGCAGAGAGT GCTGATACTG GCCTCTCTGC AGATCATGT     9599
```

FIG. 16F

H77CV-J4aa Sequence

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
MSTNPKPQRK	TKRNTNRRPQ	DVKFPGGGQI	VGGVYLLPRR	GPRLGVRATR	50
KASERSQPRG	RRQPIPKARR	PEGRAWAQPG	YPWPLYGNEG	LGWAGWLLSP	100
RGSRPSWGPT	DPRRRSRNLG	KVIDTLTCGF	ADLMGYIPLV	GAPLGGAARA	150
LAHGVRVLED	GVNYATGNLP	GCSFSIFLLA	LLSCLTIPAS	AYEVRNVSGI	200
YHVTNDCSNS	SIVYEAADVI	MHTPGCVPCV	QEGNSSRCWV	ALTPTLAARN	250
ASVPTTTIRR	HVDLLVGTAA	FCSAMYVGDL	CGSIFLVSQL	FTFSPRRHET	300
VQDCNCSIYP	GHVSGHRMAW	DMMMNWSPTT	ALVVSQLLRI	PQAVVDMVAG	350
AHWGVLAGLA	YYSMVGNWAK	VLIVALLFAG	VDGETHTTGR	VAGHTTSGFT	400
SLFSSGASQK	IQLVNTNGSW	HINRTALNCN	DSLQTGFFAA	LFYAHKFNSS	450
GCPERMASCR	PIDWFAQGWG	PITYTKPNSS	DQRPYCWHYA	PRPCGVVPAS	500
QVCGPVYCFT	PSPVVVGTTD	RSGVPTYSWG	ENETDVMLLN	NTRPPQGNWF	550
GCTWMNSTGF	TKTCGGPPCN	IGGVGNRTLI	CPTDCFRKHP	EATYTKCGSG	600
PWLTPRCLVD	YPYRLWHYPC	TLNFSIFKVR	MYVGGVEHRL	NAACNWTRGE	650
RCNLEDRDRS	ELSPLLLSTT	EWQILPCAFT	TLPALSTGLI	HLHQNIVDVQ	700
YLYGVGSAFV	SFAIKWEYIL	LLFLLLADAR	VCACLWMMLL	IAQAEAALEN	750
LVVLNAASVA	GAHGILSFLV	FFCAAWYIKG	RLAPGAAYAF	YGVWPLLLLL	800
LALPPRAYAL	DTEVAASCGG	VVLVGLMALT	LSPYYKRYIS	WCMWWLQYFL	850
TRVEAQLHVW	VPPLNVRGGR	DAVILLMCVV	HPTLVFDITK	LLLAIFGPLW	900
ILQASLLKVP	YFVRVQGLLR	ICALARKIAG	GHYVQMAIIK	LGALTGTYVY	950
NHLTPLRDWA	HNGLRDLAVA	VEPVVFSRME	TKLITWGADT	AACGDIINGL	1000
PVSARRGQEI	LLGPADGMVS	KGWRLLAPIT	AYAQQTRGLL	GCIITSLTGR	1050
DKNQVEGEVQ	IVSTATQTFL	ATCINGVCWT	VYHGAGTRTI	ASPKGPVIQM	1100
YTNVDQDLVG	WPAPQGSRSL	TPCTCGSSDL	YLVTRHADVI	PVRRRGDSRG	1150
SLLSPRPISY	LKGSSGGPLL	CPAGHAVGLF	RAAVCTRGVA	KAVDFIPVEN	1200
LGTTMRSPVF	TDNSSPPAVP	QSFQVAHLHA	PTGSGKSTKV	PAAYAAQGYK	1250
VLVLNPSVAA	TLGFGAYMSK	AHGVDPNIRT	GVRTITTGSP	ITYSTYGKFL	1300
ADGGCSGGAY	DIIICDECHS	TDATSILGIG	TVLDQAETAG	ARLVVLATAT	1350
PPGSVTVSHP	NIEEVALSTT	GEIPFYGKAI	PLEVIKGGRH	LIFCHSKKKC	1400
DELAAKLVAL	GINAVAYYRG	LDVSVIPTSG	DVVVVSTDAL	MTGFTGDFDS	1450
VIDCNTCVTQ	TVDFSLDPTF	TIETTTLPQD	AVSRTQRRGR	TGRGKPGIYR	1500
FVAPGERPSG	MFDSSVLCEC	YDAGCAWYEL	TPAETTVRLR	AYMNTPGLPV	1550
CQDHLEFWEG	VFTGLTHIDA	HFLSQTKQSG	ENFPYLVAYQ	ATVCARAQAP	1600
PPSWDQMWKC	LIRLKPTLHG	PTPLLYRLGA	VQNEVTLTHP	ITKYIMTCMS	1650
ADLEVVTSTW	VLVGGVLAAL	AAYCLSTGCV	VIVGRIVLSG	KPAIIPDREV	1700
LYQEFDEMEE	CSQHLPYIEQ	GMMLAEQFKQ	KALGLLQTAS	RHAEVITPAV	1750
QTNWQKLEVF	WAKHMWNFIS	GIQYLAGLST	LPGNPAIASL	MAFTAAVTSP	1800
LTTGQTLLFN	ILGGWVAAQL	AAPGAATAFV	GAGLAGAAIG	SVGLGKVLVD	1850
ILAGYGAGVA	GALVAFKIMS	GEVPSTEDLV	NLLPAILSPG	ALVVGVVCAA	1900

FIG. 16G

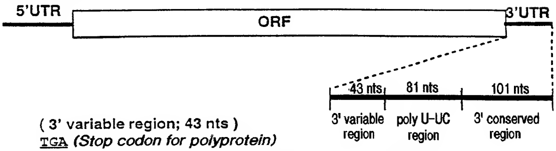
H77CV-J4aa Sequence

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
ILRRHVGPGE	GAVQWMNRLI	AFASRGNHVS	PTHYVPESDA	AARVTAILSS	1950
LTVTQLLRRL	HQWISSECTT	PCSGSWLRDI	WDWICEVLSD	FKTWLKAKLM	2000
PQLPGIPFVS	CQRGYRGVWR	GDGIMHTRCH	CGAEITGHVK	NGTMRIVGPR	2050
TCRNMWSGTF	PINAYTTGPC	TPLPAPNYKF	ALWRVSAEEY	VEIRRVGDFH	2100
YVSGMTTDNL	KCPCQIPSPE	FFTELDGVRL	HRFAPPCKPL	LREEVSFRVG	2150
LHEYPVGSQL	PCEPEPDVAV	LTSMLTDPSH	ITAEAAGRRL	ARGSPPSMAS	2200
SSASQLSAPS	LKATCTANHD	SPDAELIEAN	LLWRQEMGGN	ITRVESENKV	2250
VILDSFDPLV	AEEDEREVSV	PAEILRKSRR	FARALPVWAR	PDYNPPLVET	2300
WKKPDYEPPV	VHGCPLPPPR	SPPVPPPRKK	RTVVLTESTL	STALAELATK	2350
SFGSSSTSGI	TGDNTTTSSE	PAPSGCPPDS	DVESYSSMPP	LEGEPGDPDL	2400
SDGSWSTVSS	GADTEDVVCC	SMSYSWTGAL	VTPCAAEEQK	LPINALSNSL	2450
LRHHNLVYST	TSRSACQRQK	KVTFDRLQVL	DSHYQDVLKE	VKAAASKVKA	2500
NLLSVEEACS	LTPPHSAKSK	FGYGAKDVRC	HARKAVAHIN	SVWKDLLEDS	2550
VTPIDTTIMA	KNEVFCVQPE	KGGRKPARLI	VFPDLGVRVC	EKMALYDVVS	2600
KLPLAVMGSS	YGFQYSPGQR	VEFLVQAWKS	KKTPMGFSYD	TRCFDSTVTE	2650
SDIRTEEAIY	QCCDLDPQAR	VAIKSLTERL	YVGGPLTNSR	GENCGYRRCR	2700
ASGVLTTSCG	NTLTCYIKAR	AACRAAGLQD	CTMLVCGDDL	VVICESAGVQ	2750
EDAASLRAFT	EAMTRYSAPP	GDPPQPEYDL	ELITSCSSNV	SVAHDGAGKR	2800
VYYLTRDPTT	PLARAAWETA	RHTPVNSWLG	NIIMFAPTLW	ARMILMTHFF	2850
SVLIARDQLE	QALNCEIYGA	CYSIEPLDLP	PIIQRLHGLS	AFSLHSYSPG	2900
EINRVAACLR	KLGVPPLRAW	RHRARSVRAR	LLSRGGRAAI	CGKYLFNWAV	2950
RTKLKLTPIA	AAGRLDLSGW	FTAGYSGGDI	YHSVSHARPR	WFWFCLLLLA	3000
AGVGIYLLPN	R				3011

FIG. 16H

#1a. 3' Deletion mutants of pCV-H77C

Sequence of 3' untranslated region of pCV-H77C



(3' variable region; 43 nts)

TGA *(Stop codon for polyprotein)*

AGGTTGGGGT AAACACTCCG GCCTCTTAAG CCATTTCCTG

Afl II

(poly U-UC region; 81 nts)

TTTTTTTTTT TTTTTTTTTT TTTTTTTTCT TTTTTTTTTT CTTTCCTTTC
CTTCTTTTTT TCCTTTCTTT TTCCCTTCTT T

(3' conserved region; 101 nts)

AATGGTGGCT CCATCTTAGC CCTAGTCACG GCTAGCTGTG AAAGGTCCGT
GAGCCGCATG ACTGCAGAGA GTGCTGATAC TGGCCTCTCT GCAGATCATG
T

#1a -1. pCV-H77C(-98X) ; 3' 98 nucleotides removed from pCV-H77C

TGAAGGTTGG GGTAAACACT CCGGCCTCTT AAGCCATTTC CTGTTTTTTT
TTTTTTTTTT TTTTTTTTTT TCTTTTTTTT TTTCTTTCCT TTCCTTCTTT
TTTTCCTTTC TTTTTCCCTT CTTTAAT

#1a -2. pCV-H77C(-42X) ; 3' 42 nucleotides removed from pCV-H77C

TGAAGGTTGG GGTAAACACT CCGGCCTCTT AAGCCATTTC CTGTTTTTTT
TTTTTTTTTT TTTTTTTTTT TCTTTTTTTT TTTCTTTCCT TTCCTTCTTT
TTTTCCTTTC TTTTTCCCTT CTTTAATGGT GGCTCCATCT TAGCCCTAGT
CACGGCTAGC TGTGAAAGGT CCGTGAGCCG CAT

#1a -3. pCV-H77C(X-52) ; All of the 3' UTR sequence, except 3' 49 nucleotides, removed from pCV-H77C

TGAGCCGCAT GACTGCAGAG AGTGCTGATA CTGGCCTCTC TGCAGATCAT
GT

FIG. 17A

#1a -4. pCV-H77C(X) ; All of the 3' UTR sequence, except 3' 101 nucleotides, removed from pCV-H77C

TGAAATGGTG GCTCCATCTT AGCCCTAGTC ACGGCTAGCT GTGAAAGGTC
CGTGAGCCGC ATGACTGCAG AGAGTGCTGA TACTGGCCTC TCTGCAGATC
ATGT

#1a -5. pCV-H77C(+49X) ; The proximal 49 nucleotides of the 3' conserved region (98 nucleotides ; AAT not included) removed from pCV-H77C

TGAAGGTTGG GGTAAACACT CCGGCCTCTT AAGCCATTTC CTGTTTTTTT
TTTTTTTTTT TTTTTTTTTT TCTTTTTTTT TTTCTTTCCT TTCCTTCTTT
TTTTCCTTTC TTTTTCCCTT CTTTAATGCC GCATGACTGC AGAGAGTGCT
GATACTGGCC TCTCTGCAGA TCATGT

#1a -6. pCV-H77C(VR-24) ; First 24 nucleotides of the 3' variable region removed from pCV-H77C

TGACTTAAGC CATTTCCTGT TTTTTTTTTT TTTTTTTTTT TTTTTTTCTT
TTTTTTTTTC TTTCCTTTCC TTCTTTTTTT CCTTTCTTTT TCCCTTCTTT
AATGGTGGCT CCATCTTAGC CCTAGTCACG GCTAGCTGTG AAAGGTCCGT
GAGCCGCATG ACTGCAGAGA GTGCTGATAC TGGCCTCTCT GCAGATCATG
T

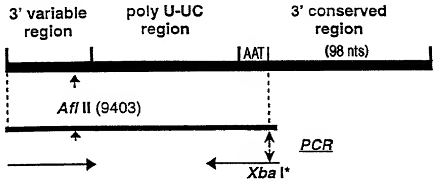
#1a -7. pCV-H77C(-U/UC) ; Poly U-UC region removed from pCV-H77C

TGAAGGTTGG GGTAAACACT CCGGCCTCTT AAGCCATTTC CTGAATGGTG
GCTCCATCTT AGCCCTAGTC ACGGCTAGCT GTGAAAGGTC CGTGAGCCGC
ATGACTGCAG AGAGTGCTGA TACTGGCCTC TCTGCAGATC ATGT

FIG. 17B

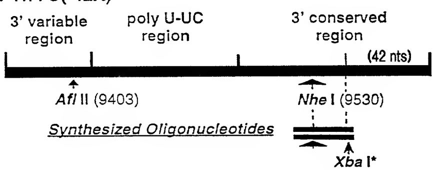
#1b. Strategy of 3' Deletion mutants

#1b -1. pCV-H77C(-98X)



1. PCR Amplification
2. Purification of PCR products
3. Digestion with *Afl* II and *Xba* I
4. Cloning of *Afl* II /*Xba* I fragment into pCV-H77C
5. Complete sequence analysis
6. in vitro transcription (within 24 hours of inoculation)
7. Percutaneous intrahepatic transfection into chimpanzee ; 11/26/97 and 12/17/97
8. Result : Negative (No replication)

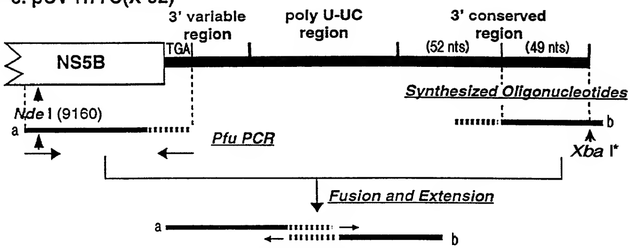
#1b -2. pCV-H77C(-42X)



1. Synthesis of oligonucleotides (sense and anti-sense)
2. Hybridization of oligonucleotides
3. Digestion with *Nhe* I and *Xba* I
4. Cloning of *Nhe* I /*Xba* I fragment into pG9-KL26 (3' UTR of H77C)
5. Sequence analysis
6. Cloning of 3' UTR (-42X) [*Afl* II /*Xba* I fragment] into pCV-H77C
7. Complete sequence analysis
8. in vitro transcription (within 24 hours of inoculation)
9. Percutaneous intrahepatic transfection into chimpanzee (Schedule; 1/22/98, 2/5/98)

FIG. 17C

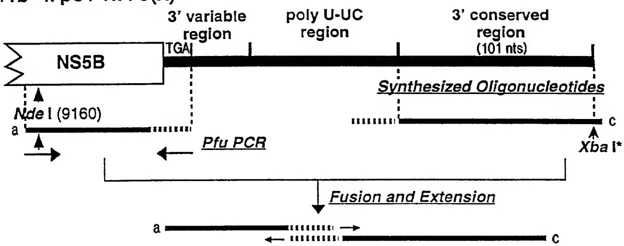
#1b -3. pCV-H77C(X-52)



1. Fragment a ; *Pfu* PCR amplification and purification
2. Fragment b ; Synthesized oligonucleotides (anti-sense)
3. Fusion and extension
4. TA cloning
5. Sequence analysis
6. Cloning *Nde* I-*Xba* I fragment with correct sequence into pCV-H77C
7. Complete sequence analysis
8. *In vitro* transcription (within 24 hours of inoculation)
9. Percutaneous intrahepatic transfection into chimpanzee

FIG. 17D

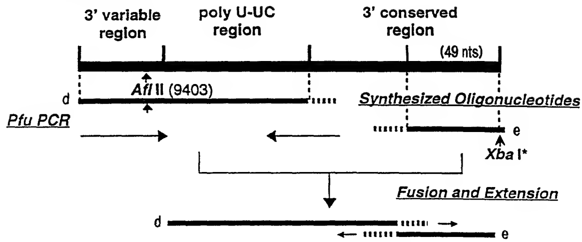
#1b -4. pCV-H77C(X)



1. Fragment a ; *Pfu* PCR amplification and purification
2. Fragment c ; Synthesized oligonucleotides (anti-sense)
3. Fusion and extension
4. TA cloning
5. Sequence analysis
6. Cloning *Nde* I-*Xba* I fragment with correct sequence into pCV-H77C
7. Complete sequence analysis
8. *In vitro* transcription (within 24 hours of inoculation)
9. Percutaneous intrahepatic transfection into chimpanzee

FIG. 17E

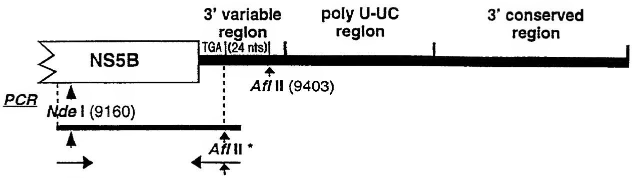
#1b -5. pCV-H77C(+49X)



1. Fragment d ; *Pfu* PCR amplification and purification
2. Fragment e ; Synthesized oligonucleotides (anti-sense)
3. Fusion and extension
4. TA cloning
5. Sequence analysis
6. Cloning *Afl* II-*Xba* I fragment with correct sequence into pCV-H77C
7. Complete sequence analysis
8. *In vitro* transcription (within 24 hours of inoculation)
9. Percutaneous intrahepatic transfection into chimpanzee

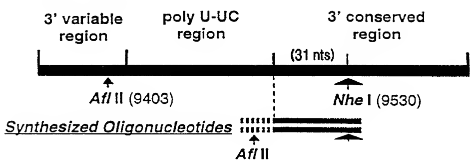
FIG. 17F

#1b -6. pCV-H77C(VR-24)



1. PCR Amplification
2. Purification of PCR products
3. Digestion with *Nde* I and *Afl* I
4. Cloning of *Nde* I /*Afl* II fragment into pCV-H77C
5. Complete sequence analysis
6. in vitro transcription (within 24 hours of inoculation)
7. Percutaneous intrahepatic transfection into chimpanzee

#1b -7. pCV-H77C(-U/UC)



1. Synthesis of oligonucleotides (sense and anti-sense)
2. Hybridization of oligonucleotides
3. Digestion with *Afl* II and *Nhe* I
4. Cloning of *Afl* II and *Nhe* I fragment into pG9-KL26
5. Sequence analysis
6. Cloning of 3' UTR (-poly U-UC) [*Afl* II /*Xba* I fragment] into pCV-H77C
7. Complete sequence analysis
8. in vitro transcription (within 24 hours of inoculation)
9. Percutaneous intrahepatic transfection into chimpanzee

FIG. 17G

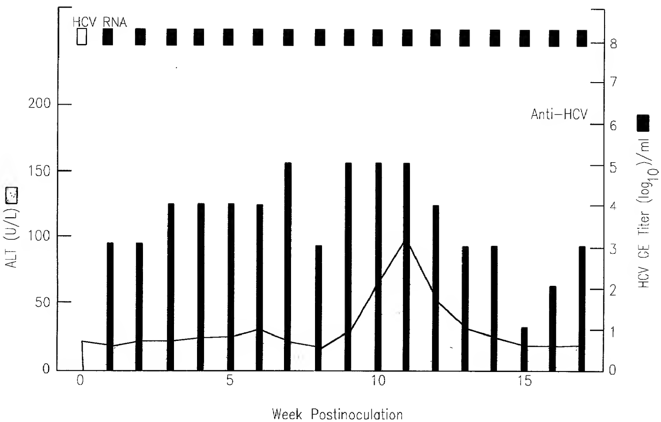

HCV RNA
Anti-HCV
ALT (U/L)
HCV GE Titer ($\log_{10}$)/ml
Week Postinoculation
0
50
100
150
200
0
5
10
15
0
1
2
3
4
5
6
7
8

FIG. 18A

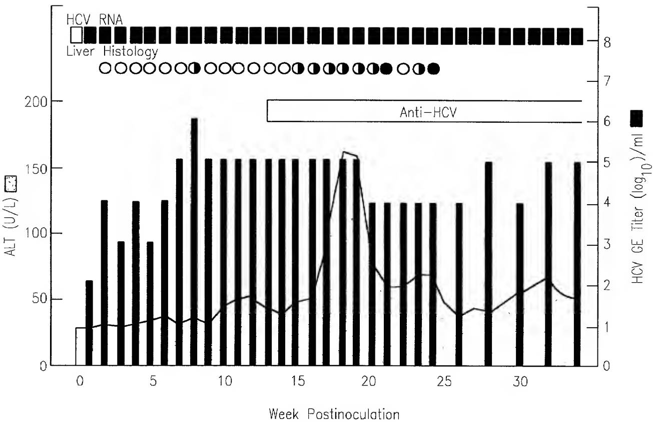

HCV RNA
Liver Histology
Anti−HCV
ALT (U/L)
HCV GE Titer $(\log_{10})$/ml
Week Postinoculation
0
50
100
150
200
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30

FIG. 18B

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, the information given herein is true, that I believe I am the original, first and sole (if only one name is listed below) or an or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: CLONED GENOMES OF INFECTIOUS HEPATITIS C VIRUSES AND USES THEREOF

which is described in:
[ ] the attached application or

[ ] PCT International Application No. ______ filed ______
[X] the specification in application Serial No. 09/014,416 filed January 27, 1998
(if applicable) and amended on ______

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known to me which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56 (a).

I hereby claim foreign priority benefits under Title 35 United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign applications(s) for patent or inventor's certificate or any PCT international applications(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

COUNTRY	APPLICATION	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC § 119
			[ ] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) or PCT International application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

Application Serial No.	Filing Date	Status: patented, pending, abandoned

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States Provisional patent application(s).

Provisional Application Serial No.	Filing Date	Status: patented, pending, abandoned
60/053,062	18 July 1997	Pending

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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Office of Technology Transfer
National Institutes of Health
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Rockville, MD 20852
Telephone: (301) 496-7056
Fax: (301) 402-0220

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature: M. Yanagi Date: 3/19/98

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Inventor's signature: Jens Bukh Date: 3/19/98

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Full Name of third joint inventor: Suzanne U. Emerson

Inventor's signature: Suzanne U. Emerson Date: 3/19/98

Country of Citizenship: United States of America

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Full Name of fourth joint inventor: Robert H. Purcell

Inventor's signature: Robert H. Purcell Date: 3/19/98

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Post Office Address: 17517 White Grounds Road, Boyds, Maryland 20841, U.S.A.

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Emerson, Susanne U.
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Bukh, Jens

<120> CLONED GENOMES OF INFECTIOUS HEPATITIS C VIRUSES AND
USES THEREOF

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35 40 45

Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
50 55 60

Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly
65 70 75 80

Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp
85 90 95

Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
100 105 110

Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys
115 120 125

Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu
130 135 140

Gly Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp
145 150 155 160

Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile
165 170 175

Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr
180 185 190

Gln Val Arg Asn Ser Ser Gly Leu Tyr His Val Thr Asn Asp Cys Pro
195 200 205

Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Ala Ile Leu His Thr Pro
210 215 220

Gly Cys Val Pro Cys Val Arg Glu Gly Asn Ala Ser Arg Cys Trp Val
225 230 235 240

Ala Val Thr Pro Thr Val Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr
245 250 255

Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys
260 265 270

Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Ser Val Phe Leu Val Gly
275 280 285

Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr Thr Gln Asp Cys
290 295 300

Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp
305 310 315 320

Asp Met Met Met Asn Trp Ser Pro Thr Ala Ala Leu Val Val Ala Gln
325 330 335

Leu Leu Arg Ile Pro Gln Ala Ile Met Asp Met Ile Ala Gly Ala His
340 345 350

Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser Met Val Gly Asn Trp
355 360 365

Ala Lys Val Leu Val Val Leu Leu Leu Phe Ala Gly Val Asp Ala Glu
370 375 380

Thr His Val Thr Gly Gly Asn Ala Gly Arg Thr Thr Ala Gly Leu Val
385 390 395 400

Gly Leu Leu Thr Pro Gly Ala Lys Gln Asn Ile Gln Leu Ile Asn Thr
405 410 415

Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu Asn Cys Asn Glu Ser
420 425 430

Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr Gln His Lys Phe Asn
435 440 445

Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Arg Leu Thr Asp
450 455 460

Phe Ala Gln Gly Trp Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly Leu
465 470 475 480

Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro Cys Gly Ile
485 490 495

Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser
500 505 510

Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro Thr Tyr Ser
515 520 525

Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg Pro
530 535 540

Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe
545 550 555 560

Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly Val Gly Asn
565 570 575

Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala
580 585 590

Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro Arg Cys Met
595 600 605

Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile Asn Tyr
610 615 620

Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg Leu
625 630 635 640

Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp
645 650 655

Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Gln Trp
660 665 670

Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr Gly
675 680 685

Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly
690 695 700

Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu Tyr Val Val
705 710 715 720

Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ser Cys Leu Trp
725 730 735

Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu Asn Leu Val
740 745 750

Ile Leu Asn Ala Ala Ser Leu Ala Gly Thr His Gly Leu Val Ser Phe
755 760 765

Leu Val Phe Phe Cys Phe Ala Trp Tyr Leu Lys Gly Arg Trp Val Pro
770 775 780

Gly Ala Val Tyr Ala Leu Tyr Gly Met Trp Pro Leu Leu Leu Leu Leu
785 790 795 800

Leu Ala Leu Pro Gln Arg Ala Tyr Ala Leu Asp Thr Glu Val Ala Ala
805 810 815

Ser Cys Gly Gly Val Val Leu Val Gly Leu Met Ala Leu Thr Leu Ser
820 825 830

Pro Tyr Tyr Lys Arg Tyr Ile Ser Trp Cys Met Trp Trp Leu Gln Tyr
835 840 845

Phe Leu Thr Arg Val Glu Ala Gln Leu His Val Trp Val Pro Pro Leu
850 855 860

Asn Val Arg Gly Gly Arg Asp Ala Val Ile Leu Leu Met Cys Val Val
865 870 875 880

His Pro Thr Leu Val Phe Asp Ile Thr Lys Leu Leu Leu Ala Ile Phe
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Gly Pro Leu Trp Ile Leu Gln Ala Ser Leu Leu Lys Val Pro Tyr Phe
900 905 910

Val Arg Val Gln Gly Leu Leu Arg Ile Cys Ala Leu Ala Arg Lys Ile
915 920 925

Ala Gly Gly His Tyr Val Gln Met Ala Ile Ile Lys Leu Gly Ala Leu
930 935 940

Thr Gly Thr Tyr Val Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala
945 950 955 960

His Asn Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe
965 970 975

Ser Arg Met Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala Ala
980 985 990

Cys Gly Asp Ile Ile Asn Gly Leu Pro Val Ser Ala Arg Arg Gly Gln
995 1000 1005

Glu Ile Leu Leu Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp Arg
1010 1015 1020

Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu
1025 1030 1035 1040

Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu
1045 1050 1055

Gly Glu Val Gln Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala Thr
1060 1065 1070

Cys Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg
1075 1080 1085

Thr Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val
1090 1095 1100

Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser Leu
1105 1110 1115 1120

Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His
1125 1130 1135

Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu
1140 1145 1150

Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro
1155 1160 1165

Leu Leu Cys Pro Ala Gly His Ala Val Gly Leu Phe Arg Ala Ala Val
1170 1175 1180

Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Asn
1185 1190 1195 1200

Leu Gly Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro
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Pro Ala Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr
1220 1225 1230

Gly Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly
1235 1240 1245

Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe
1250 1255 1260

Gly Ala Tyr Met Ser Lys Ala His Gly Val Asp Pro Asn Ile Arg Thr
1265 1270 1275 1280

Gly Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr
1285 1290 1295

Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile
1300 1305 1310

Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly
1315 1320 1325

Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val
1330 1335 1340

Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Ser His Pro
1345 1350 1355 1360

Asn Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr
1365 1370 1375

Gly Lys Ala Ile Pro Leu Glu Val Ile Lys Gly Gly Arg His Leu Ile
1380 1385 1390

Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val
1395 1400 1405

Ala Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser
1410 1415 1420

Val Ile Pro Thr Ser Gly Asp Val Val Val Val Ser Thr Asp Ala Leu
1425 1430 1435 1440

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1445 1450 1455

Cys Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile
1460 1465 1470

Glu Thr Thr Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg
1475 1480 1485

Gly Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro
1490 1495 1500

Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys
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Val Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln
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Asp His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile
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Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Phe Pro
1570 1575 1580

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1585 1590 1595 1600

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1825 1830 1835 1840

Ser Val Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly
1845 1850 1855

Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu
1860 1865 1870

Val Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser
1875 1880 1885

Pro Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg
1890 1895 1900

His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile
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Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro
1925 1930 1935

Glu Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Ser Leu Thr
1940 1945 1950

Val Thr Gln Leu Leu Arg Arg Leu His Gln Trp Ile Ser Ser Glu Cys
1955 1960 1965

Thr Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile
1970 1975 1980

Cys Glu Val Leu Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met
1985 1990 1995 2000

Pro Gln Leu Pro Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Arg
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Val Ser Phe Arg Val Gly Leu His Glu Tyr Pro Val Gly Ser Gln Leu
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Asp Pro Ser His Ile Thr Ala Glu Ala Ala Gly Arg Arg Leu Ala Arg
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Gly Ser Pro Pro Ser Met Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala
2195 2200 2205

Pro Ser Leu Lys Ala Thr Cys Thr Ala Asn His Asp Ser Pro Asp Ala
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2245 2250 2255

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2260 2265 2270

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2275 2280 2285

Ala Arg Pro Asp Tyr Asn Pro Pro Leu Val Glu Thr Trp Lys Lys Pro
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Asp Tyr Glu Pro Pro Val Val His Gly Cys Pro Leu Pro Pro Pro Arg
2305 2310 2315 2320

Ser Pro Pro Val Pro Pro Pro Arg Lys Lys Arg Thr Val Val Leu Thr
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Asp Ser His Tyr Gln Asp Val Leu Lys Glu Val Lys Ala Ala Ala Ser
2485 2490 2495

Lys Val Lys Ala Asn Leu Leu Ser Val Glu Glu Ala Cys Ser Leu Thr
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Thr Arg Lys Ala Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
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Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Leu Gly Trp Ala Gly Trp
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Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
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Glu Val Arg Asn Val Ser Gly Ile Tyr His Val Thr Asn Asp Cys Ser
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Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Val Ile Met His Thr Pro
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Thr Arg Lys Ala Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
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Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Ala Trp Ala Gln Pro Gly
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Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Leu Gly Trp Ala Gly Trp
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Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
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Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys
115 120 125

Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu
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Gly Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp
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Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile
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Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Ile Pro Ala Ser Ala Tyr
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Glu Val Arg Asn Val Ser Gly Ile Tyr His Val Thr Asn Asp Cys Ser
195 200 205

Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Val Ile Met His Thr Pro
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Gly Cys Val Pro Cys Val Gln Glu Gly Asn Ser Ser Arg Cys Trp Val
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Thr Ile Arg Arg His Val Asp Leu Leu Val Gly Thr Ala Ala Phe Cys
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Ser Ala Met Tyr Val Gly Asp Leu Cys Gly Ser Ile Phe Leu Val Ser
275 280 285

Gln Leu Phe Thr Phe Ser Pro Arg Arg His Glu Thr Val Gln Asp Cys
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Asn Cys Ser Ile Tyr Pro Gly His Val Ser Gly His Arg Met Ala Trp
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Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val Val Ser Gln
325 330 335

Leu Leu Arg Ile Pro Gln Ala Val Val Asp Met Val Ala Gly Ala His
340 345 350

Trp Gly Val Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp
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Ala Lys Val Leu Ile Val Ala Leu Leu Phe Ala Gly Val Asp Gly Glu
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Thr His Thr Thr Gly Arg Val Ala Gly His Thr Thr Ser Gly Phe Thr
385 390 395 400

Ser Leu Phe Ser Ser Gly Ala Ser Gln Lys Ile Gln Leu Val Asn Thr
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Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser
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Leu Gln Thr Gly Phe Phe Ala Ala Leu Phe Tyr Ala His Lys Phe Asn
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Ser Ser Gly Cys Pro Glu Arg Met Ala Ser Cys Arg Pro Ile Asp Trp
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Phe Ala Gln Gly Trp Gly Pro Ile Thr Tyr Thr Lys Pro Asn Ser Ser
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Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala Pro Arg Pro Cys Gly Val
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Val Pro Ala Ser Gln Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser
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Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Val Pro Thr Tyr Ser
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Trp Gly Glu Asn Glu Thr Asp Val Met Leu Leu Asn Asn Thr Arg Pro
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Pro Gln Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe
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Thr Lys Thr Cys Gly Gly Pro Pro Cys Asn Ile Gly Gly Val Gly Asn
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Arg Thr Leu Ile Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala
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Thr Tyr Thr Lys Cys Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys Leu
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Ser Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg Leu
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Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Glu Trp
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Gln Ile Leu Pro Cys Ala Phe Thr Thr Leu Pro Ala Leu Ser Thr Gly
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Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly
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Val Gly Ser Ala Phe Val Ser Phe Ala Ile Lys Trp Glu Tyr Ile Leu
705 710 715 720

Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ala Cys Leu Trp
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Met Met Leu Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val
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Val Leu Asn Ala Ala Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe
755 760 765

Leu Val Phe Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Ala Pro
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Gly Ala Ala Tyr Ala Phe Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu
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His Pro Thr Leu Val Phe Asp Ile Thr Lys Leu Leu Leu Ala Ile Phe
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Gly Pro Leu Trp Ile Leu Gln Ala Ser Leu Leu Lys Val Pro Tyr Phe
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Val Arg Val Gln Gly Leu Leu Arg Ile Cys Ala Leu Ala Arg Lys Ile
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Ala Gly Gly His Tyr Val Gln Met Ala Ile Ile Lys Leu Gly Ala Leu
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Thr Gly Thr Tyr Val Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala
945 950 955 960

His Asn Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe
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Ser Arg Met Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala Ala
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Cys Gly Asp Ile Ile Asn Gly Leu Pro Val Ser Ala Arg Arg Gly Gln
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Glu Ile Leu Leu Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp Arg
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Gly Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr
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Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile
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Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val
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Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Ser His Pro
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Gly Lys Ala Ile Pro Leu Glu Val Ile Lys Gly Gly Arg His Leu Ile
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Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val
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Ala Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser
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Val Ile Pro Thr Ser Gly Asp Val Val Val Val Ser Thr Asp Ala Leu
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Met Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr
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Cys Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile
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Glu Thr Thr Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg
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Val Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln
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Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Phe Pro
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Pro Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro
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Thr Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln
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Asn Glu Val Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Thr Cys
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Met Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly
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Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val
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Val Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro
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Asp Arg Glu Val Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ser
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Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe
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Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg His Ala Glu
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Val Ile Thr Pro Ala Val Gln Thr Asn Trp Gln Lys Leu Glu Val Phe
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Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala
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Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala
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Phe Thr Ala Ala Val Thr Ser Pro Leu Thr Thr Gly Gln Thr Leu Leu
1795 1800 1805

Phe Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Ala Pro Gly
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Ala Ala Thr Ala Phe Val Gly Ala Gly Leu Ala Gly Ala Ala Ile Gly
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Ser Val Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly
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Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu
1860 1865 1870

Val Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser
1875 1880 1885

Pro Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg
1890 1895 1900

His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile
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Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro
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Glu Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Ser Leu Thr
1940 1945 1950

Val Thr Gln Leu Leu Arg Arg Leu His Gln Trp Ile Ser Ser Glu Cys
1955 1960 1965

Thr Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile
1970 1975 1980

Cys Glu Val Leu Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met
1985 1990 1995 2000

Pro Gln Leu Pro Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Arg
2005 2010 2015

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Tyr Thr Thr Gly Pro Cys Thr Pro Leu Pro Ala Pro Asn Tyr Lys Phe
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Ile Gln Leu
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Pro Tyr Cys

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Ile Gln Leu
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Ile Gln Leu
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<210> 45
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Ile Gln Leu
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PATENT
Docket No. 2026-4276

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Masayuki Yanagi, et al.

Serial No. : 09/014,416 Group Art Unit: To be assigned

Filed : January 27, 1998 Examiner: To be assigned

For : CLONED GENOMES OF INFECTIOUS HEPATITIS C VIRUSES AND USES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231

ASSOCIATE POWER OF ATTORNEY

Dear Sir:

Pursuant to the provisions of 37 CFR 1.33 and 1.34 and MPEP 402.02, the undersigned attorney of record hereby appoints the following as associate attorneys to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office in connection with the above-identified application:

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Respectfully submitted,

Date 20 March 1998

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Assistant Commissioner for Patents
Washington, D.C. 20231

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